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BIOLOGICAL BULLETIN

STUDIES IN THE LIFE CYCLE OF SIMOCEPHALUS VETULUS.

WYMAN REED GREEN,
HULL ZOÖLOGICAL LABORATORY.

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I. INTRODUCTION.

These studies on *Simocephalus* were begun at the Hull Zoölogical Laboratory of the University of Chicago, in 1913. The life cycle is very complex and has not been worked out satis-

factorily in all of its phases. Although very creditable work has been done by Chambers ('13) on spermatogenesis in *Simocephalus*, and by Weismann and a host of others on the natural history, oogenesis, fertilization, etc., of the Daphnians in general, there is still urgent need of more data. Much time and effort have been expended by the writer in the attempt to perfect a technique which would be adequate to the cytological problems. Considerable progress has been made with this phase of the work yet the study is far from completion. The present paper will be devoted to some problems naturally arising in the course of such studies, which should be solved before or at least in coördination with the cytological problems, else the latter would lose much of their significance.

Among such problems are the following: the normal general life cycle must be known; whether this may not be modified by altering the external conditions and if so, to what extent and how; whether the species may not express itself in all of its forms under each of several sets of conditions, or if there are certain environmental factors which bear special relations to certain forms; whether the failure to produce sexual forms during the parthenogenetic phase is due to a deficiency in the environmental complex or to internal conditions such as age or is dependent simply upon the general rate of metabolism; the normal proportions of males and females; the ratio of sexual[1] to asexual females, the sequence of broods; the cause of senescence of the cultures; the cause of the appearance of the ephippial eggs; the time in the ontogeny at which they appear; the precise function of these eggs, *i.e.*, whether the importance of the ephippial egg is centered in the egg as a means of tiding the species over unfavorable seasons or periods, or in the stem mother developing from it; the relation of the males to the production and development of the ephippial eggs; the normal length of the latent period; if it be of definite duration; whether it can be shortened and if so, how; whether the offspring of a stem mother

[1] By the term "sexual female" in this paper we designate those females producing a series of ephippial eggs which require fertilization; by "asexual female" those which reproduce only parthenogenetically. All so-called sexual females are destined to pass from their sexual phase into the parthenogenetic phase and so remain.

are different from the offspring of females produced parthenogenetically; whether any difference is to be found between the offspring of parthenogenetic females which have passed through a sexual period and those which have not; whether a sexual female may not be retained in the sexual state, etc.

This paper presents data accumulated chiefly during the four years from 1914 to 1918 at Carleton College biological laboratory, Northfield, Minn., bearing upon the solution of these and other problems and definite conclusions regarding some of them. There naturally arises the very interesting and important problem of ascertaining just what correlations exist between the general course and the cytological aspects of the life cycle. The details of my work on this latter phase of the problem are to be given in another paper, now under way.

II. Literature Review.

The theory that there is a sex cycle in Cladocera which is independent of external factors was formulated by August Weismann. It is now most certainly known that, although the correlation existing between environmental factors and the production of parthenogenetic and sexual forms is not exact, certain environmental complexes do exist which completely inhibit the appearance of males and sexual females.

A review of the very extended work of Weismann (1877–87) and his contemporaries such as Kurz (1875) and Schmankewitsch (1877), and of later writers as Issakowitsch (1907), of Kuttner (1909), Woltereck (1909), Papanicolau (1911) and others, leaves one very unsettled as to what is the general determinative principle underlying the course of events in the normal life cycle of even the well known and much studied genera such as *Moina*, *Daphnia*, and *Simocephalus*. The Weismannian conception of a relatively fixed "generation cycle" for *Cladocera* is well known, and has been adopted wholly or in a modified form by practically all writers up to about 1914, although its truth had been questioned by several experimenters. About this time some results were obtained by several workers using various species of the three genera mentioned, which must be interpreted as positive evidence against the theory. Since Weismann's

time the recorded opinions range all the way from unconditional acceptance of his extreme view to a complete lack of faith in it. For convenience of discussion these various views may, in general, be grouped under two chief heads:

1. That external factors are of little or of no importance in relation to the succession of periods of parthenogenetic and sexual reproduction. This was the conviction of Weismann, based upon his researches extending over a long period of years. This theory was broad enough to cover all forms of life in which there is a succession of parthenogenetic and sexual reproduction. Accordingly the evidence for the theory extends over a wide field, and it has numerous supporters, having been for a long time the orthodox view.

This general view is supported by Keilhack (1906) and Ekman (1905) working on *Polyphemus*, by Popoff (1907) working on various protozoa and metazoa, and by Punnett (1906) and Whitney (1907) working on *Hydatina*, and by others. The last two authors concluded that the age of the strains is a weighty factor in causing the appearance of the sexual forms. The conclusions of Strohl (1908) constitute a clean cut statement of the views up to that time. Working with *Polyphemus*, he concluded that there was no reason for abandoning the well grounded views of Weismann. Kuttner (1909) working on *Simocephalus vetulus* and other Daphnians adopted Weismann's extreme view. McClendon (1910) considers that "The life cycle of a Daphnid is, therefore an heredity tendency, but can be influenced by nutrition and probably by temperature and the accumulations of excretions," and he adds "Nutrition is the most important factor."

The extended experiments of Woltereck (1909–11) on *Cladocera* led him to admit automaticity as one of the factors in determining the course of their life cycle. Assuming that Woltereck's cultural conditions (1911, fig. 4, p. 152) were kept the same in all four cultures for the full four years, one would be forced to the conclusion that there are periods when environmental factors are of no influence, and possibly other periods when they are, since the four cultures responded not only differently in different years, but different cultures responded differently in the same years; in other words, the conclusion would seem to follow that

there is autonomous variation in this respect, which places the whole question beyond the pale of possible experimental proof. The careful experiments of Papanicolau (1911) merit more consideration than is admissible in this paper. The views of Issakowitsch (1907) and Scharfenberg (1911) agree with those of Woltereck and Papanicolau in that they all assume certain inherent tendencies to sexuality which cannot be completely overcome by any kind of environmental conditions.

2. The alternative view is that outer causes, such as chemical substances, hunger, temperature, kind of food, etc., are largely or entirely responsible for the varying degrees of sexual and parthenogenetic reproduction. Evidence for this heterodox view had begun to accumulate at least as early as 1875, when Kurz noted a correlation between the drying up of the water and the appearance of sexual forms in *Cladocera*. Two years later Schmankewitsch (1877) gave as his opinion that the efficient cause is the increasing salt concentration due to evaporation. Among the unfavorable conditions mentioned by Kerhervé (1892, p. 236) poor nourishment is particularly emphasized as being responsible for the appearance of both males and sexual females. Ostwold (1094) found temperature singularly effective as a cause of sexuality in Daphnids. By varying the temperature he produced at the same time all of the forms that are found in nature at different seasons. Langhans (1909, p. 291) says that Weismann's theory of a fixed generation cycle will not bear critical examination.

In regard to other forms whose life cycle is in general similar to that of *Cladocera*, there is much diversity of opinion, although evidence against a fixed internal "cycle" is rapidly accumulating.

Finally we must include under this second category the very significant and definite conclusions of Grosvenor and Smith (1913) working with *Moina rectirostris*, of Banta (1914) working with *Daphnia pulex*, and of Agar (1914) working with *Simocephalus vetulus*, each of whom has conclusively demonstrated that, for the form experimented on, there are certain environmental complexes which will indefinitely inhibit the appearance of males and sexual states in any of the females. It is of interest to note that in some instances the conditions which will thus prevent the full expression of the species is quite narrowly prescribed.

A third category of opinion is sometimes tacitly implied by the manner in which the problem of the succession of forms in *Cladocera* is discussed by those authors who believe that the primary causes are cytological. Were this true the problem would not of course be thereby removed from the domain of possible influence by environmental factors. Here should be mentioned the well-known "kern-plasma" theory of Hertwig. It is supported by Papanicolau, Issakowitsch, Popoff and others.

The results of Von Scharfenberg and Papanicolau have been brought by Child (1915) into relation with certain phases of his general theory of organic constitution, as developed in his book, entitled "Senescence and Rejuvenesence." He says (p. 391): "Von Scharfenberg and Papanicolau found that a change in egg character occurred, not only in the course of successive generations, but also in the course of single generations, *i.e.*, the eggs produced early in the life of a female are more likely to develop parthenogenetically into females and those produced later in life into males or to be zygogenic winter eggs. In the earlier generations of a cycle the male producing and zygogenic eggs appear later in the life of the individual, in later generations earlier." I am convinced that the conception that zygogenic eggs normally arise in Daphnians *after* the parthenogenetic eggs is based on entirely insufficient evidence. Definite statements bearing on this point will be found in Papanicolau's papers (1910, p. 740, and 1911, p. 82). These views are in substantial agreement with those of Issakowitsch (1907) and Scharfenberg (1911), and diametrically opposed to my own since I have never found an instance of the production of ephippial eggs following a period of parthenogenetic reproduction. I am convinced that these conclusions could not have been based upon observations on isolated individual females of *Simocephalus vetulus*. Grosvenor and Smith (1913, p. 514) working on *Moina rectirostris* state: "We did not find any case of a female that had produced eggs parthenogenetically turning into an ephippial female."

For several years the writer was not able to duplicate the results of those experimenters who claimed that, under certain conditions, reproduction in some Cladocera will proceed for an indefinite number of generations parthenogenetically, and that

the change to sexual reproduction, together with the degeneration that so often accompanies it under experimental conditions, are wholly dependent upon the environment. Later experimentation however (experiment 5), has demonstrated their contention to be correct, at least for *Simocephalus vetulus*. Though these last experiments are of a very different type from those described by Agar (1914*b*, Table I.), Grosvenor and Smith (1913), and Banta (1914), who succeeded in carrying Daphnians through many generations with no loss of vigor, they are corroborative of their results. That the success of these authors in rearing purely parthenogenetic generations for an indefinite time could not have been due to the accidental selection of lines with a strong internal tendency to parthenogenesis is rendered certain not only by the use of several distinct lines, but also by parallel cultures subjected separately to unfavorable temperatures and crowding, which gave a large proportion of males and sexual females. The genera used were *Simocephalus*, *Moina* and *Daphnia*. Banta carried *Daphnia pulex* for 127 generations with no loss of vigor.

In groups other than Cladocera in which it has been found that an indefinite number of generations can be reared parthenogenetically, it seems that it has always been possible to bring on sexuality by a proper change in the environment, which would indicate that pure lines with respect to parthenogenesis do not exist. It now seems altogether likely that bisexuality may be indefinitely inhibited in many more of the lower animals than experimenters have been aware of heretofore. The conditions under which this is possible have been found in some instances to be narrowly circumscribed, as *e.g.*, certain Cladocera require the high temperature of 28° C., although the more recent authors seem to believe that a considerable number of species of Cladocera will be found which can be induced to reproduce by parthenogenesis indefinitely if only the external conditions are properly manipulated. It appears also that, whatever may be the explanation, any set of nearly uniform conditions finally becomes prejudicial to continued parthenogenesis. Parthenogenetic reproduction seems to be favored by conditions which are conducive to rapid growth, though they may vary within certain limits.

Each species of Cladocera found capable of pure parthenogenesis has its own specific requirements as to which environmental factors may vary and to what extent without it becoming zygogenic.

III. Material.

On taking up this work much difficulty was encountered in rearing algæ as food for the Daphnians. Numerous formulæ were tried with varying success, but for some reason they did not thrive on algæ artificially produced. A simple and satisfactory solution of the difficulty was discovered in the following method: A number of three- or four-gallon aquaria nearly full of water are placed where they will not receive the direct rays of the sun and stocked with several kinds of unicellular algæ. One to three frogs, depending upon the size, which have been in captivity until little or nothing remains in the alimentary canal, are placed in each of the jars. Of course the aquaria must be covered, in part to retain the frogs, but the more important reason for this is to insure the maintenance of a high percentage of carbonic acid gas and the depletion of oxygen. Under such conditions the algæ grow rapidly until a condition of equilibrium is reached. When a sufficient quantity of algæ has developed a single large Daphnian with brood pouch full of eggs or embryos may be introduced and the frogs removed, or one may be left in with good results. When the Daphnians have overstocked the culture they may be strained out with a cloth and transferred to a new jar. The brown sediment of the old culture, which is excreta and dead algæ, should be carefully removed so as not to loosen the algæ on the sides of the jar, new water added and more algæ grown in the manner indicated above.

The above method has served me very satisfactorily for *Simocephalus vetulus*, though it is not so well adapted to some other forms. *Daphnia pulex* thrives in water which is more or less filled with putrescent matter. In a pond frequented daily by cattle and thus kept very roily and malodorous, I found this species in such numbers that by a single dip of the net I secured hundreds of them. For best results one needs to work out special methods for each species. In all of my work I have used *Simocephalus vetulus* unless otherwise stated.

IV. Isolation Experiments.

In order to ascertain the course of events in the normal general life cycle I isolated numerous specimens, at random at first, and kept a complete record of all of their offspring. Some of these will be presented in detail. For convenience all experiments will be referred to by number.

Experiment 1.—On June 23 a very large female with the brood pouch full of embryos was isolated. On June 24 a brood of 47 asexual females was extruded. These embryos were saved and a first brood secured from each of 25 of them. The remainder died early. There were 13 pure broods of males, the numbers in the broods being as follows: 4, 3, 1, 3, 1, 1, 1, 2, 2, 2, 6, 2, and 7. There were 6 mixed broods as follows: 3 males and 6 females, 8 males and 4 females, 6 males and 6 females, 1 male and 6 females, 5 males and 3 females, and 2 males and 4 females. The numbers of females in the pure broods of females were: 4, 5, 6, 3, 4 and 2. Now with this single instance before us we might well ask why this great variety in the offspring of the members of a pure brood of 47 asexual females? But in the light of further experimentation along this same line we may reasonably suppose that had the mother of these 47 female embryos lived to produce other broods, some would have consisted of males or at least would have contained males.

Another female isolated at the same time lived until July 13, when she died with embryos in her brood pouch. Her series of broods is as follows: 1 male and 6 undetermined, 7 females, 15 males, 50 females and 1 male, 15 males, 9 females, and 13 females. The 50 females of the mixed brood were saved and the first brood of each of 19 of them was secured. There were 12 broods of males containing 8, 12, 3, 8, 12, 3, 12, 4, 5, 41, 12 and 6. There was one mixed brood of 12 males and females, and 6 pure broods of females contained 6, 10, 1, 3, 2 and 6.

There seemed to be no constancy as to the ratio of males to females in the offspring of these isolated females, nor in the sequence of broods, some producing males first, some females, and others mixed broods, in a most capricious manner. Hence these miscellaneous experiments demonstrated the desirability of much more extended experimentation to discover if possible what order might underlie this apparent confusion.

Experiment 2.—The following experiment was designed to show in particular the normal ratio of males to females, and their sequence, though it furnishes data bearing upon several other points. It was begun on June 23 by the isolation of a single female from a laboratory culture. She produced the following broods on the dates indicated·

On June 26 a brood of 43, 27 being female, 16 not living.

On June 28 a brood of 49 females.

On July 3 a brood of 75 females.

On July 7 a brood of 7 males.

On July 10 a brood of 6 females.

On July 11 the isolated female died.

Of the brood produced on June 28, 45 which lived were saved for experiment. These were isolated and the sex of all of their offspring determined, and in several instances the kind of females *i.e.*, whether sexual or asexual. The members of this brood are numbered. The date of the first broods of each of the first 17 was July 4, of all of the remainder it was July 5. The date of the death of each female is given at the end of her series of broods. The date of the last brood is practically always not more than one day before the death of the female. The second brood of female number 1 is a mixed brood and they are so indicated throughout.

1. 9 females, 5 females and 9 males, 9 females, an undetermined brood, July 15.

2. 9 females, a mixed brood of about 20 containing many dead, 6 females, July 12.

3. 4 females, 3 males and 6 undetermined, 11 males, 4 females, July 13.

4. 4 females, 18 males and 2 females, 6 females, 1 female, July 13.

5. 3 females, 11 males and 8 females, 16 females, July 9.

6. 9 males, 3 undetermined, 6 females, 22 females, July 10.

7. 7 females, 7 females and 8 undetermined, 6 females, 5 females, an undetermined brood, July 14.

8. 6 females, 3 females and 10 undetermined, 10 males, July 7, 14.

9. 8 females, 2 males, 4 males, July 9.

10. 6 females, 3 males and 16 undetermined, July 7.

11. 6 males and 1 undetermined, 4 males and 1 female, 4 females, an undetermined brood, July 13.

12. 8 males, 3 males, 3 females, an undetermined brood, July 9.

13. 7 males, 1 male, 7 females, July 9.

14. 9 females, a mixed brood undetermined, 6 females, July 12.

15. 6 females, 9 males, 7 females, July 8.

16. 10 males, 6 males, 5 females and 4 undetermined, 10 females, July 9.

17. 9 females, 38 females, 10 males, July 15.

18. 12 females, 7 females and 6 males, 10 undetermined, 7 females, July 9. All of the last brood of 7 females were asexual but some of their offspring were sexual.

19. 6 females, 10 males and 14 undetermined, 7 females, 14 females, July 9.

20. 8 females, 21 males, 8 females, two other undetermined broods, July 10.

21. An undetermined brood, 11 males and 12 females and 14 undetermined, July 9.

22. 7 females, 12 males and 7 females, an undetermined brood, July 9.

23. 10 males, 2 males and 13 females, 3 females, July 13.

24. 4 males, 6 asexual females, 10 females, 2 sexual females, 15 males, 24 females of which 2 were sexual and 2 asexual and 8 males, (the other 20 females of the last brood died too early for their sexuality to be determined), 13 females 7 of which were asexual and 6 undetermined, 16 asexual females and 1 male, 2 females 1 being asexual and 1 sexual, 22 females 12 being sexual and 10 undetermined, the next two broods were accidentally left together but their sum was 35 females of which 12 were sexual and 11 asexual while the remaining 12 were undetermined, the final brood was 7 females of which 5 were sexual 1 asexual and 1 undetermined, August 2.

25. 5 females, 20 males, 4 females, July 9.

26. One male and 5 females, 11 males and 2 females, 7 females, July 10.

27. 4 males, 1 male and 4 females, July 7.

28. 7 females, 12 males, 3 sexual females, 7 males, 1 male, July 12.

29. 5 males, 7 males, 6 males, 2 males and 11 females, July 14.

30. 5 females, 8 males and 9 undetermined, July 8.

31. 4 males, 8 males, July 7.

32. 8 females, 16 males, 1 male, July 9.

33. 2 females, 22 males, 8 females, July 9.

34. 6 males, 7 males, July 8.

35. 6 males, 5 males, July 8.

36. 8 males, 5 males, 4 females, July 9.

37. 8 females, 7 males, July 8.

38. 5 females, 22 females, 6 males, an undetermined brood, 20 females, 1 male and 35 females, July 15.

39. 19 males, an undetermined brood, 15 females, July 8.

40. 4 females, July 7. (mother with abnormal carapace, offspring normal).

41. 3 males, 3 males, 6 females, 7 males and 7 sexual females, 5 males and 2 sexual females, 1 sexual female, 1 female and 5 undetermined, 20 females 15 of which are asexual the other 5 may be, 15 females, 17 females, 3 females 2 of which are sexual and 1 asexual, 2 females 1 of which is asexual the other undetermined, 9 females 7 of which are asexual 1 sexual and 1 undetermined, July 31.

42. 6 males, 2 males, 13 females, 1 male and 2 females, July 13.

43. 5 males and 2 females, 11 males, 7 females, 11 males, 1 male, 6 females, 11 males and 1 female, 2 males, a brood of females, 5 females, an undetermined brood, 1 female, July 26.

44. 7 males, 8 males, 6 males, 2 males, 10 females, 9 females, 24 undetermined, 9 females, 8 females, 12 females, an undetermined brood, 4 females, 4 asexual females, August 4.

45. 8 females, 23 males, an undetermined brood, 8 males, 8 males, 15 males, 10 females, 18 females, 1 male and 2 females, 1 sexual and 1 asexual, an undetermined brood, 7 females, July 24.

SUMMARY OF EXPERIMENT 2.

The number dying without offspring	4
" " possibly producing no males	3
" " " " " females	2

The percentage of males is about	42
The number of times the first brood was purely female	26
" " " " " " " " " male	16
" " " " " " " " a mixed brood	2
" " " " " " " " undetermined	1
" " " " " last " " purely female	28
" " " " " " " " " male	11
" " " " " " " a mixed brood	6
The total number of mixed broods	22
The number producing no mixed broods	25
" " of times the broods immediately preceding and following mixed broods are of the same sex	9
The number of times they are of a different sex	4
The average number in each pure first brood of females	6.6
" " " " " " " " " males	7
" " " " " mixed brood	16
" " " of offspring per individual	39
" " " " broods " "	4.3
" " " " hours between the broods	45.3
" " " " female broods per individual	2.1
" " " " male " " "	1.3
" " " " females in each female brood	4.5
" " " " males " " male "	11.9
" " " " " produced per individual	15.5
" " " " females " " "	20.7
" " " " days each of the 45 females lived	14
The total number of offspring was slightly over	1705

The 45 females under consideration in this experiment were divided into three groups. The first group, consisting of 24 individuals, was kept in water taken from an "asexual" culture and were well fed; the second group of 20 were also well fed but were kept in water taken from a culture which was producing an abundance of males and sexual females; the third group of 5 being reared in the same kind of water as the second but were poorly fed. The results of this part of the experiment are given in the following table in which the data have been reduced

	Group 1	Group 2	Group 3
Average length of life in days	12.4	11.0	27.2
" number of broods	3.6	3.0	10.6
" " " female broods	2.1	1.1	5.2
" " " females produced	21.6	12.3	43.4
" " " males "	11.5	15.6	29.2
" " " male broods	.9	1.4	3.2
" " " offspring	37.6	26.6	80.6
	%	%	%
Mixed broods	9	10	11

to the basis of the individual, the data on the first, second, and third groups being given in the first, second, and third columns respectively, of the table.

Comparing these three groups we find that the differences when averaged are not great enough to be significant. The members of the third group lived roughly two and one half times as long as the others, and should we reduce the data to a common unit of time the correspondence would be very close. For instance, the number of broods produced by the first group per unit of time, say 27 days, would be 10 ($2.25 \times 3.6 = 10.00$), while the third group produces just 10.6. The number of female broods produced by the first group would be 4.72 ($2.25 \times 2.1 = 4.72$), as against 5.2 broods produced by the third group, etc. As to all of the main points under consideration the figures agree so closely that we must conclude that the kind of water in which the three groups were reared and the relative food supply had little or nothing to do with the ratio of sexes. In the general discussion of this experiment further data are given based on the observation of isolated females whose ancestry is now known for one or two generations, with conclusions regarding the relation of sexual to asexual females, males to ephippial egg production, etc. It is deemed better however, to defer this until after the presentation of another general isolation experiment in which stem mothers were used instead of females selected from the general cultures.

Experiment 3.—Although most of my experiments to induce ephippial eggs to hatch have been failures I have given them in detail further on in this paper because of the fact that so little has been accomplished along this line. I have succeeded in securing about 70 stem mothers and have had a fair degree of success in rearing them. All of these were isolated at once and a complete record was kept of the number of broods, the kinds of individuals in each brood, length of life, etc., as shown in the table below. It was found that a large number died early, producing few or no offspring. A few however produced as many as 9 broods. The average number of broods produced per stem mother was slightly over 6. Hence in the tabulation of results no account was taken of stem mothers whose broods were too few,

since these were probably not perfectly normal, else they would have lived longer, nor of broods beyond the sixth except to consider them in determining the average number of broods per stem mother, since the averages were not materially changed by eliminating them. Accordingly only 24 stem mothers were considered fit to include in the final averages, and for similar reasons the number of mothers in the F_2 and F_3 generations were reduced to 16 and 12 respectively. I was much surprised to note the large proportions of males and sexual females in the broods of these stem mothers. This observation suggested the experiment of rearing several generations to discover the relative proportions of males and sexual females in the first and later generations, to compare the offspring of the stem mothers with that of the females produced parthenogenetically, and to learn what difference, if any, is to be found between the offspring of sexual females after they have passed through the sexual state and of those females which never pass through this state. It seemed that even if passing through the sexual state did not affect the ratio of the sexes, or of asexual to sexual females, in the immediate offspring, that the effect might conceivably be cumulative and would be apparent in the succeeding generations. Hence the sexual and asexual females in the F_1 generation were segregated and two distinct lines, one sexual and the other asexual, were carried to the F_3 generation. Perhaps the most conspicuous feature in the records of this experiment is one shown only by the individual records of the females, namely that they are variable in the extreme. A given stem mother may produce nearly all males, or one kind of females, for several broods, or throughout her life, or they may appear combined in all proportions, just as is shown in the detailed individual records given in experiment 2, female number 41. The most important facts however are to be deduced from the summary given at the end of the tabulated results. In order to facilitate the comparison of results in the sexual and asexual lines in the second and third generations, I have placed the figures in juxtaposition and have reduced them to a percentage basis at the end of the table, besides giving the chief data in simpler form following the main summary of this experiment. Of course the results in all cases

are somewhat in error because of the number of offspring which die too early for identification; but the error is not considerable since all of the available evidence points to the conclusion that all of the different kinds of offspring are about equally viable. Inspection of this table shows no marked changes in the relative number of kinds of offspring from the first to the later broods in any of the generations in either the sexual or the asexual lines. This would corroborate the conclusion already reached from the numerous other experiments, namely that sex is in no way correlated with the age of the mother. It was of the greatest interest to find that the offspring of the F_3 generation in the sexual line were no more likely to be males and sexual females than were the offspring of the F_3 generation in the asexual line. It seems that if such selection were to have any definite effect that it would at least begin to show by the third generation. Yet it will be observed that in the second generation 33.3 per cent. of the offspring in the asexual line is male, and 32.7 per cent. is male in the sexual line; while in the third generation the corresponding percentages are 35 per cent. and 31.5 per cent. respectively. Of the stem mothers the percentage of males in the offspring is 23.5 per cent., the lowest of all. It seems to me however that the differences in no case are great enough to be of any significance in a species of animals which displays so much variation in so many respects as this one does.

The ratio of the asexual to the sexual females is seen to be 27.6 per cent.: 23.1 per cent. in the F_1 generation; 19.1 per cent.: 15.3 per cent. in the F_2 generation of the asexual line, 21.9 per cent.: 11.3 per cent. in the F_2 generation of the sexual line; 20.7 per cent.: 17.6 per cent. in the F_3 generation of the asexual line, and 28.2 per cent.: 12 per cent. in the F_3 generation of the sexual line. The last ratio is striking as showing such a low percentage of sexual females in the sexual as compared to the asexual lines in the same generation.

It will be seen that if the percentages of the males in these five categories are averaged we get somewhat over 31 per cent., which is very much lower than that observed in experiment 2, which was 42 per cent. Since in the latter case the offspring considered numbered over 1,700, it may seem that we should be

justified in concluding that the percentage of males in the generations immediately following the stem mothers is normally low. Such a conclusion would be erroneous, since several experimenters have succeeded in maintaining cultures of *Simocephalus* and other genera indefinitely in the parthenogenetic phase. In all cases in my experiments where females were isolated and kept in small containers in the laboratory some of their broods were sure to contain sexual forms if the mothers lived to produce a reasonable number. In the experiment under discussion the sexual forms did not increase up to the sixth brood, after which so large a proportion of stem mothers died that it seemed best to close the experiment. However at this point experiment 5 is complementary since it is concerned with a large number of females which are of varying degrees of remoteness from stem mothers and we find them producing only about five per cent. of sexual forms. It seems quite clear that the remoteness of the generation from the stem mother bears no definite relation to the numerical ratio of male to female offspring or of sexual to asexual females.

As to the degree of sexuality of the sexual females concerned in this experiment I find no evidence that it is different from that observed in the former experiment. The number of ephippia produced can doubtless be taken as a crude index to the degree of sexuality of a female, since, as shown elsewhere, the number produced is dependent upon factors inherent in the female, fertilization of the ephippial egg having no relation whatever to the continuance of the sexual state. The number of ephippia cast by each sexual female in this experiment averages slightly less than two. I have found very few females which produce as many as five ephippia. Only three instances are recorded in all of my experiments. The production of three is common. Here as in all other cases observed, the sexual individuals passed the sexual state by the time they were about half to two thirds grown, and never returned to it once they had become asexual. The only observer so far as I am aware recording the contrary for *Simocephalus vetulus* is Issakòwitsch (1908). Moreover, ephippia which were barely noticeable would sometimes appear and be cast, asexuality coming on at once. Other individuals would develop their ephippia to half or two thirds the normal size and

TABULATED SUMMARY OF EXPERIMENT 3.

Description of the Five Categories.	Offspring of the Stem Mothers, *i. e.*, the F1 Generation.						The F2 Generation. Asex. Line is Upper Figures. Sex. Line is Lower Figures.						The F3 Generation. Asex. Line is Upper Figures. Sex. Line is Lower Figures.					
Kinds of Offspring in these Categories so Far as Known.	Totals.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex not Known.	Totals.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex not Known.	Totals.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex not Known.
Number of mothers	24						16						12					
							16						12					
Average number of broods from each	6.4						6.0						5.5					
							8.2						6.2					
Average time of bearing, in days	28.1						23						19					
							31						20					
Average length of life, in days	36.1						30						29					
							39						32					
Average number of kinds of offspring	15.7	4.2	7.6	.6	1	2.3	10.3	.6	0	6	1.5	2.2	7	1	2	4	0	0
of 1st broods							6.2	1.6	.1	1.5	2	1	9.7	2	.9	3.8	.4	2.6
Average number of kinds of offspring	14.1	2.2	5.1	2.1	1.8	2.9	8.5	3	2.1	1.4	0	2	5	1.5	1	2	.5	0
of 2d broods							5.5	1.7	1.8	.7	1.3	0	8.1	3	0	2.2	1	1.9
Average number of kinds of offspring	9.4	1.6	1.7	3.1	1	2	11.1	2.1	2.3	3.1	2.5	1.1	8	2.1	1.8	1.1	2	1
of 3d broods							8.7	1.3	.4	4	3	0	6.5	.6	1.2	1.7	3	0
Average number of kinds of offspring	12.4	6.2	1	4.5	.4	.3	4.7	1.6	.3	2.1	.7	0	7.5	2.1	.9	2.5	2	0
of 4th broods							6.9	.7	0	2.2	4	0	11	5	2	2.1	1.9	0
Average number of kinds of offspring	11.8	4.5	.8	4.3	1.7	.5	6.6	1.2	.3	2	1	2.1	4.7	.5	1.3	1.9	0	1
of 5th broods							7.5	1	2	3	1.5	0	12.2	4.2	1	4	2	1
Average number of kinds of offspring	7.1	1	.1	1	5	0	5.6	.5	2.2	1	1.6	.3	7	.9	0	2.1	4	0
of 6th broods							4	2	0	2	0	0	12.1	2	2	5	3.1	0
Sum of the six broods	70.5	19.7	16.3	16.6	10.9	8	46.8	9	7.2	15.6	7.3	7.7	39.2	8.1	7	13.6	8.5	2
							37.8	8.3	4.3	12.4	11.8	1	59.6	16.8	7.1	18.8	11.4	5.5
Percentage of females in all six	66.1						50						59.9					
broods							64.4						59.3					
Average number of kinds of offspring	11.7	3.2	2.7	2.7	1.8	1.3	7.8	1.5	1.2	2.6	1.2	1.3	6.5	1.3	1.1	2.2	1.4	.3
of all broods							6.3	1.4	.7	2	2	.2	9.9	2.8	1.2	3.1	1.9	.9
Totals reduced to a percentage basis	100	27.6	23.1	23.5	15.4	11.3	100	19.1	15.3	33.3	15.6	16.1	100	20.7	17.6	35	21.6	5.1
							100	21.9	11.3	32.7	31.2	2.6	100	28.2	12	31.5	19.1	9.2

become asexual. After the production of several ephippia a partial one may be developed as the female leaves the sexual state, or she may change over abruptly. The degrees of sexuality are infinite, since it is subject to continuous and not to integral variation.

Considering the stem mothers from the standpoint of size attained, viability, length of life, activity, etc., in the first few generations of offspring, in addition to the proportion of the kinds of offspring as to sex, and the degree of sexuality of the females, one is forced to conclude that stem mothers are not functionally at all unlike the females which are produced parthenogenetically, with the exception that not a single instance of the production of an ephippial egg by a stem mother has been noted. This last is in strict accordance with the findings of Grosvenor and Smith (1913) for the stem mothers of *Moina rectirostris*, and of most writers, though rarely instances of the contrary are noted, *e.g.*, Scharfenberg (1911, p. 24).

The most important data are excerpted from the tabulated summary of experiment 3 and slightly rearranged in the following table for the convenience of the reader.

Kinds of Offspring.	Asexual Females.	Sexual Females.	Males.	Other Females.	Sex Not Known.
Percentage of kinds of offspring (F_1 gen.) in the first 6 broods of 24 stem mothers.....	27.6	23.1	23.5	15.4	11.3
Percentage of kinds of offspring (F_2 gen.) in the first 6 broods of 16 F_1 asexual females	19.1	15.3	33.3	15.6	16.1
Percentage of kinds of offspring (F_2 gen.) in the first 6 broods of 16 F_1 sexual females after becoming parthenogenetic.....	21.9	11.3	32.7	31.2	2.6
Percentage of kinds of offspring (F_2 gen.) in the first 6 broods of 12 F_2 asexual females	20.7	17.6	35	21.6	5.1
Percentage of kinds of offspring (F_2 gen.) in the first 6 broods of 12 F_2 sexual females after becoming parthenogenetic.....	28.2	12	31.5	19.1	9.2

Experiment 4. Temperature experiments with *Simocephalus* yield very indefinite results. Individuals do not thrive at a higher temperature than 28° C. Even at this temperature isolated individuals live only a short time. Several experiments designed to test the effect of high temperature were performed in the following manner. Specimens were taken from laboratory cultures and placed in glass containers having loose covers to

prevent excessive evaporation. In each of these experiments four containers were used, of about one pint capacity, two thirds full of water at room temperature when the Daphnia were introduced. Each vessel contained a sufficient quantity of green algæ for 25 females. These containers were placed in an electric parafin bath and the temperature maintained at 28° C. The first one or two broods produced under high temperatures were found to contain the usual proportions of males and the two kinds of females (see tabulated summary of experiment 3). These first broods were discarded. Thus all of the offspring in the tabulated results of one of these experiments given below, passed their entire ontogeny at a temperature of 28° C.

Dates on Which Offspring were Removed.	Total Number of Mothers Living on Dates Indicated.	Offspring.				
		Asexual Females.	Sexual Females.	Males.	Other Females.	Sex Not Known.
August 25	106	21	0	28	16	3
August 30	102	14	12	30	42	17
September 5	81	6	10	11	38	8
September 7	17	2	0	7	5	8
September 10	3	0	3	2	0	4
September 11	0					
Total number of kinds of offspring		43	25	78	101	41
Percentages		14.9	8.7	27.0	34.0	14.0
Total number of offspring						288

The most important point to be noted in these results is that all kinds of offspring continued to appear to the last. Although the percentages vary somewhat, they are within the limit of normal variation. Other experiments were performed to determine the effect of lower temperatures. All of the kinds of offspring were secured at a temperature of 14° C., but the general metabolism is so much lowered at this temperature that the individuals were too few to justify any conclusions as to the ratio of the sexes. Both low and high temperatures, since they lower the rate of metabolism, induce a decrease in the average size of the broods, in the number of broods, viability of the offspring, and shorten the lives of the females used in experimenting. In this connection it is of interest to note that females which were reproducing parthenogenetically have been obtained every

month in the year from shallow ponds near Gary, Ind. The offspring obtained in the winter months were asexual females. While no sexual females and males were obtained from eggs and embryos borne by females at the time of collecting from under the ice, the offspring thus secured were not sufficiently numerous to justify any statement as to the production of males and sexual females at this season. I am uncertain as to just how far these experimental results hold true in nature, but there seems to be no reason for considering temperature a vital factor in relation to the sex cycle of *Simocephalus vetulus*. As mentioned elsewhere, Grosvenor and Smith (1913) completely inhibited sexual forms in *Moina rectirostris* at a temperature of 28° C., but since they could not decide as to what factor their success was due it is probably safe to assume that they succeeded in spite of the high temperature, not because of it.

Experiment 5.—The following experiment was performed to discover what kinds of offspring are produced under natural conditions. It involves 51 females collected at various times from April 20 to May 25, in the vicinity of Northfield, Minn., from three widely separated permanent freshwater ponds. The sizes of the females ranged from small (hence young) to very large (hence old) individuals. The largest female among the lot was slightly over 4 mm. from the anterior margin of the head to the posterior end of the carapace and $2\frac{5}{6}$ mm. in vertical measurement. There is no doubt that the age and size of *Simocephalus* correspond very closely. Though I know the pedigree and brood records of none of these 51 females it may be assumed on the basis of size that some, such as the smallest, had produced very few broods, while others, the larger ones, had produced very many, probably 15 or 20. Although the individuals were not isolated there was no crowding. They were placed in two-quart fruit jars, no jar containing more than six individuals. Jars containing several specimens were kept full. They were placed outside the laboratory windows where the sun would not strike them, being thus subject to the ever varying temperatures. Only water brought from the ponds where the females were collected was used. This was strained through a fine silk cloth which removed all metazoa, but allowed

the passage of small unicellular algæ. After the first few days a gradually thickening film of green algæ grew on the sides of the jars. To insure that slight accumulations of excreta and other waste might not affect them the water was changed every two days. No food was provided other than that which was suspended in the water and which passed through the fine strainer. They were thus subjected to the same culture medium, food and the same night and day temperatures, as they were in the natural environment. It was thus hoped to duplicate as nearly as possible the natural conditions of these ponds and to acquire positive evidence as to the kind of offspring being produced under natural conditions by testing only the first broods produced after the females were collected. It will be noted that the tabulated presentation of this experiment gives the dates of the collecting trips in the first column, the number of specimens secured on each trip in the second column, the dates on which all young were separated from their mothers and placed in other similar jars in the third column, the number of offspring removed on each date in the fourth, the number of offspring which proved to be female in the fifth, etc., Records were kept of more than one brood from the females collected on May 17, May 20 and May 25, and it is interesting to note that the fourth brood produced by the single female collected on May 17 consisted of 50 males and one sexual female. In calculating the percentages of kinds of offspring only those first removed were taken into consideration. Of the 524 offspring in this category none is positively known to be male and 98.8 per cent. are positively determined to consist of females. All of the first offspring of the 19 females collected on April 20, May 16 and May 23 were saved to test for sexuality. It is seen that 95.6 per cent. were asexual, unless possibly there were more than 7 sexual females in the brood of 77 produced by the single very large female collected on May 16. Most of this brood died very early and the mother died also without further progeny. Excluding this one female's offspring the percentage of asexual offspring of the other 18 females is over 99 per cent. No sexual forms, either male or female, were collected, but a few ephippial eggs were secured by skimming the surface of the ponds and these on dissection seemed to be

perfectly fresh, and could hardly have been unhatched eggs of the preceding season. That they were *Simocephalus* eggs is quite certain since the only other Daphnian of similar size in these ponds is *Daphnia pulex*, whose ephippia contain two eggs each.

That the practical freedom from sexual forms in this instance is due to external conditions is rendered absolutely certain by the fact that these same females when the jars were placed in the laboratory and the water no longer regularly changed, begun to produce males and sexual females, in just about the same proportions as in experiment 3 and 4, as nearly as could be judged from general observation.

TABULATED SUMMARY OF EXPERIMENT 5.

Date Collected.	Number of Mothers.	Date of Removal of Broods.	Number of Offspring.	Females.	Males.	Asexual Females.	Sexual Females.	Sex Unknown.
April 20	4	1st April 28	33	33	0	33	0	0
May 6	11	1st May 8	45	45	0	?	?	0
May 9	6	1st May 11	15	15	0	?	?	0
May 16	1	1st May 17	77	77	0	?	7	?
May 17	1	1st May 20	4	?	?	?	?	4
		2d May 23	15	15	0	15	0	0
		3d May 25	45	45	0	45	0	0
		4th May 30	51	1	50	0	1	0
		5th June 3	34	34	0	?	?	0
May 20	6	1st May 23	?	?	?	?	?	?
		2d May 26	120	118	2	?	?	0
May 23	14	1st May 25	150	150	0	149	1	0
May 25	8	1st May 30	200	198	?	?	?	2
		2d June 3	101	97	1	?	?	3
Totals.....	51		890	828	53	242	9	9
Totals in 1st broods.....			524	518	0	182	8	6
Percentages of kinds of offspring in the 1st broods.....			100	98.8	0	95.6	4.3	?

V. EXPERIMENTS WITH EPHIPPIAL EGGS.

Authors are not agreed as to the factors involved in shortening the latent period of the ephippial egg. Experimentation along this line has yielded the most inconsistent results. I have repeated the experiments of Weismann and others of freezing the eggs for varying lengths of time with all but complete failure. Hence I shall give in detail the experiments I have performed in

my attempts to discover a means of ending the latent period, although in not a single instance have results been all that could be desired. In a considerable number of my experiments I have made special effort to reproduce the factors operating upon these eggs in nature, by a series of freezings, alternated with drying, placing the eggs in the sunlight, etc., but the results seem to indicate that I have neglected the one thing needful. Of one thing I am quite certain, namely, that the fact that the eggs of *Simocephalus vetulus* can be collected from the fresh-water ponds every month in the year must be taken into account in formulating any general conclusions as to the factors concerned with their development. Unless otherwise stated, eggs of *Simocephalus vetulus* were used in the following experiments. In experiments performed with eggs collected from the field there were usually several kinds present but those of *Simocephalus* predominate. My only successes have been with *Simocephalus* eggs which were produced in the laboratory.

Experiment 1.—Since the eggs that are laid in ponds are, as the ponds dry up, subjected to gradualllly increasing concentrations of whatever salts may be in solution in the pond water it occurred to me that this might be a potent factor in terminating the latent period. To test this I took numerous ephippial eggs from my laboratory cultures and placed them in a pint of pond water and allowed it to stand exposed in the laboratory in a wide stender dish. I placed a graduated scale on the side of the dish, dividing the water depth into ten equal spaces. When the water had lowered to each of the various levels by evaporation, I took out a number of the eggs and placed them in covered vessels. Continuing thus until the water in the open dish had all disappeared, I left the last lot in the open vessel until it was thoroughly dry. The same experiment was tried starting with low concentrations of NaCl, but the results were negative in all cases.

Experiment 2.—A large number of ephippial eggs were collected from the surface of the ice on temporary ponds near Gary, Ind., on March 12, 1914, and dried at once. On June 20, 1916, they were placed in water at room temperature and left. They were watched for several months until it seemed

certain that none would develop. On August 15 the experiment was discontinued. If freezing is the most important factor as some authors have believed, it certainly cannot be all sufficient, for this lot of eggs numbered several hundreds and they were frozen up in the ice for many weeks before they were transferred to water. At two later dates other lots were collected and treated similarly. The last was on March 21, when the ice had begun to thaw.

One lot of eggs taken from a laboratory culture in April were dried for three days, then placed in water at room temperature but did not hatch. After two months they were taken out and dried. Over two years later they were again transferred to water at room temperature without success. These experiments were performed at the University of Chicago, but before leaving there I secured great numbers of ephippial eggs by skimming the surface of the ponds near Gary, Ind., and at intervals of every few months thereafter for three years placed a number of these in water. None ever hatched. The eggs treated in this way were not counted though 1,000 would be a very conservative estimate.

Experiment 3.—This experiment was performed with ephippial eggs which had been recently produced in the laboratory cultures. They were subjected to very low concentrations of H_2SO_4, namely, M/100, M/10, M/5, M/2 and M/1, for 1, 2, 10, 24 and 72 hours. Three eggs were used for each separate set of conditions. The eggs were transferred to water and examined daily. Results were negative. A precisely similar experiment was then carried out with KOH in the place of the H_2SO_4, without success.

Experiment 4.—On January 15, 1914, 10 ephippial eggs were taken from a laboratory culture and dried for three hours, then kept in a freezing mixture for eight hours after which they were transferred to water at room temperature. None hatched.

On the same date 6 ephippial eggs were transferred directly from a laboratory culture to water which was kept at 30° C. for four weeks. Another lot was kept at 28° C. Results were negative in both cases.

On January 18, 1914, some ephippial eggs which had been

produced in the latter part of December, 1913, in laboratory cultures, were subjected to the following conditions: One lot of 7 was subjected to a freezing mixture for eight hours, one lot of 6 to a freezing mixture for eight hours on two successive days, and another lot of 6 were given the same treatment on three successive days. In each case the eggs were transferred from the freezing mixture to water at room temperature. The same kind of treatment was applied to large numbers of eggs which had been collected by scraping the surface of the ice on fresh water ponds near Gary, Ind., permitting the eggs to dry between the freezings. Others were given the same treatment and then dried for three months before being transferred to water at 14° C., but all to no avail.

Experiment 5.—During the course of my work I have made numerous attempts to induce development by clipping off the small ends of the ephippia with scissors or scalpel, by pricking the shell with a needle, and by dissecting it completely off. It is hardly to be supposed that such treatment would have any effect upon eggs that had never been dried, since they often have the ventral edges of the ephippia only lightly apposed, it being possible in some instances to look between them upon the egg inside, but conceivably desiccated eggs might be thus influenced. It is quite a simple matter to spread the valves and set the egg free. I have succeeded in three instances in placing ephippial eggs with the shells removed in the brood pouch of asexual females. This did not incite development.

On August 14, 1916, I dissected the shells from 18 ephippial eggs and pricked them with an extremely fine glass needle. In some cases I barely pierced the vitelline membrane, and in others pushed the needle about one third the way through the egg. The membrane is quite tough and in most cases is under considerable tension due to osmotic pressure. There is a quantity of liquid underlying the membrane and minute quantities of this can be seen to exude from the wound in all cases, and if the wound is made with too large a needle or too deeply the granular cytoplasm is extruded. Presumably such eggs are destroyed. Fourteen of these eggs seemed to have been successfully treated and were placed in a stender dish with enough

algæ to nourish any embryos that might appear. They were examined daily for some weeks and at intervals for two months. Some of the eggs degenerated early and others persisted as if no injury had been received. None had developed on October 10, and shortly thereafter no eggs could be found.

Experiment 6.—On January 15, 1916, two dozen eggs were taken from a laboratory culture which had been producing an abundance of them, and without being allowed to dry, were placed in a vessel where the sun would strike them for a few hours daily, for some weeks. They were then placed out of the sun. On August 16 I placed them on ice for 24 hours, and then left them until June, 1917. That they were in perfect condition at this time was shown by the fresh green color of the eggs on removal of the ephippia. They were held under water by being placed in a vial which was left lying on its side in a stender dish. None of these developed.

Experiment 7.—On June 30, 1916, a number of ephippial eggs of *Daphnia pulex* were taken from a laboratory culture and placed under water in the same manner as in the above experiment and left thus submerged until May 20, 1917. Dissection of the ephippia from several of these showed them to be in good condition at the end of this time, none having developed.

Experiment 8.—Although all of my carefully devised attempts to induce ephippial eggs to hatch by the application of chemical and mechanical stimuli, as well as the numerous repetitions of the freezing experiments of Weismann, have completely failed, I have succeeded in securing about 70 stem mothers from some *Simocephalus* vetulus eggs which had been given no special treatment. On June 27, 1916, I removed several hundred eggs from a culture which had produced great numbers of them and placed them in a large-mouthed 8 oz. bottle lying in a horizontal position in a one-gallon battery jar about one third full of water. A small amount of unicellular green algæ was added. Cypris of several species soon appeared in large numbers. On August 14 an embryo of *Simocephalus* appeared. I removed it in a little of the same water together with some of the algæ. It lived only three days. Fortunately others were to follow. On August 18 another appeared, and produced a brood of 7 females

on August 24. Eight more hatched on August 21, 7 more on August 25, 10 on August 26, 6 on August 30, 4 on September 3, 2 September 6, 1 September 20, and at more or less irregular intervals for several months thereafter others continued to appear, until nearly six dozen had been secured.

Two other instances deserve mention. Three days before setting up this experiment I had placed a number of eggs, produced from May 1, to June 26, in a jar in a similar manner and they remained there until December 12, when they suddenly began to hatch, but only a few appeared. I can suggest no explanation as to why they did not all hatch, or why the few that did should not have done so earlier as did most of those mentioned above. On August 9 some fresh eggs were placed on ice and left 36 hours and then placed under water in the manner described above. On December 27 two hatched. No more appeared although the experiment was left standing until June 1, 1917, *i.e.*, about 11 months.

Mortality among these stem mothers was found to be about the same as with other females. I succeeded in obtaining an average of a little over six broods from each of 24 of these stem mothers and have given their history in the interesting experiment (number 3) already described.

It should be stated that there were numerous other eggs in the culture from which the majority of the stem mothers was secured, which did not hatch. Some of the water from this culture was removed and other newly produced eggs placed in it. This experiment was left standing from August 30, 1916, until May 5, 1917. Since none of these developed we must infer that the kind of water in the battery jar containing the developing eggs, was not the responsible factor, though I have not the remotest idea what it may have been.

VI. Observations on Pairing.

A female from a culture containing many ephippial females was isolated on July 19. By July 27 she had produced two female broods, one of 14 and another of 10. I placed the older brood with males. On August 1st I found that 14 ephippia had been produced, though 10 of them were empty, and all but one of

the females had become asexual. The one sexual female remained so until she had developed three ephippia. Then she developed a brood of 5 asexual females and died August 9. It was not surprising to find so large a percentage of empty ephippia appearing in the presence of males since the same thing occurs in general cultures, though I had thought it might, in the latter case, be due to too small a percentage of males. That this explanation is erroneous is shown by this and the following experiments.

Several of the female broods obtained from the two females isolated in isolation experiment number 1, were placed with numerous males and examined daily. On July 1 more males were added since I noticed several incipient ephippia appearing. On July 30 the culture numbered about 150 individuals, and only two ephippia had fully developed, one of these being empty. By August 6 there had been developed 14 ephippia, most of them empty. The percentage of ephippial females thus remained less than 10 per cent., so low that it is quite obvious that the presence of males could not be the factor responsible for their appearance, since larger proportions of sexual females often occur in females which are segregated from the males. Not all of the ephippia reached full size, though most of them did. There was a dearth of males at no time during this experiment, yet less than half of the fully developed ephippia contained fertilized eggs. The fourth brood of female number 18 of isolation experiment 3, consisted of 7 females. These when very young were placed with 10 males. All seven, however, proved to be asexual, although some of their offspring developed into sexual females in the absence of males.

The 20 females of the fifth brood of female number 38, of isolation experiment 2, were divided into two lots, 10 being kept with males and 10 without. Of the 10 with males, 9 developed ephippia, a few of which contained eggs when the molt was cast. These eggs, however all degenerated, so presumably they had not been fertilized. The males had all died at this time. Six of these females became asexual after producing the first ephippium. I placed some more males with the remaining four sexual females and was fortunate enough to observe two matings of

the same female within two hours. I saw no spermatozoa lost in these instances as I have in several cases observed later, and I have no doubt that the one normal ephippial egg which I found later was the result of this pairing.

Of the 10 without males, one died early, 5 developed ephippia, and 4 became asexual at once. The 5 sexual females developed in all 16 ephippia, which is more, and more per individual, than was produced by those kept with males. Males appeared in some of the broods of the asexual females, but most of the ephippia were produced before they appeared, and there was only one fertilized egg. The sexual females all became asexual before death.

The 35 females of the sixth brood of this same female number 38, were also saved. The one male of the brood died at the age of four days. Of the 16 ephippia produced by the members of this brood one contained an egg. Since the egg did not degenerate it must have been fertilized. Thus we have proof of the early functioning of the males. They are shorter lived than the females, or at least that has been the case with individuals isolated. It will be noted that this brood contained three kinds of individuals, namely asexual females, sexual females, and a male. My records contain numerous instances of this kind.

All of the females of broods 4, 5, 8 and 11 of female number 41 of isolation experiment 3, were placed with males and a complete record kept of the results. The fourth brood consisted of 7 males and 7 females, all sexual. In all 17 ephippia were cast by these 7 females. Two of them then died and the remainder became asexual. In spite of the presence of the males 15 out of these 17 ephippia contained no egg. The fifth brood consisted of 5 males and two sexual females. These were reared together. Each female produced a fertilized egg and one of them an empty ephippium afterward, this one then dying. The other became asexual though the males were continually present. The eighth brood consisted of 20 females. A number of males were placed with these. Five of the females died early, and none of the rest developed even an incipient ephippium. The eleventh brood consisted of 3 females, 2 being sexual and 1 asexual. The

asexual one was discarded. These two females whose mother was asexual were reared under conditions which were as nearly alike as they could possibly be made and yet have them in two separate vessels, gave rise to diverse offspring. An equal number of males was placed with each. The history of one was: an empty ephippium, 4 males, 4 sexual and 2 asexual females, an undetermined brood, death. The history of the other was: an empty ephippium, a fertilized egg, a fertilized egg, 3 sexual and 3 asexual females, 1 sexual and 4 asexual females, death. Each produces an empty ephippium at first, but the former then produces a brood of 4 males, and the latter another ephippium, this time containing a fertilized egg. Both later produce both kinds of females. Several other experiments of this same kind gave similar results. I wish to point out here only one conclusion based on these experiments, namely, that all afford evidence that the reason for the production of male broods is not the production of ephippial eggs which were not fertilized because of the absence of males, for in these experiments the onset of male brood production has been observed many times in the females which have passed from the sexual state and begun the production of males, in the presence of other males.

The method of copulation is interesting. This was observed in several instances. The sixth brood of female number 44, of isolation experiment 2, consisted of 8 sexual females and 1 asexual female. When the first ephippia of these 8 sexual females were in their incipiency I introduced 5 males and watched them almost continuously, removing them when I could not give them attention. When the females were about 6 days old the ephippia were well developed and the ephippial eggs seemed to be ready to be extruded into the brood pouch. It was at this time that the monotony of proceeding was relieved. On being placed in the vessel with the females the males at once became intensely excited. This lasted nearly an hour. After several abortive attempts at pairing one pair mated. The male opened his carapace slightly and clasped the antero-ventral margin of one side of that of the female. He then bent his abdomen ventrad, so that it extended between the ventral margins of the female's carapace, ventral to her posterior appendages. In

this position he waved his abdomen about for a few seconds, then straightening it, he ejected the spermatozoa in two forcible streams, which, like puffs of smoke, spread abruptly when their force was spent. They seemed to me to be all swept away by the exhalant respiratory current of the female. The excitement of the males was now abated, and in about fifteen minutes I removed the males. Two hours later I introduced the males again and secured a second mating. In this instance the male ejected the spermatozoa just anterior to the posterior end of the abdomen of the female, and posterior to her last pair of appendages. Again I saw some of the sperm cells swept away, but very few. I am now convinced that the first case described was abortive. Other observations were made later and in some cases no part of the mass of spermatozoa was lost, though no case was observed in which the sperm cells were not ejected freely into the respiratory chamber as described, the only point of contact being the ventral margins of the male's carapace with the antero-ventral margin of one side of that of the female. The ephippial egg is laid within about an hour, and in no case observed more than two hours, after the pairing. During the period of œstruation the female doubtless produces some chemical substance which passes out in her exhalent respiratory current, since it is by passing through this that the males are made aware of the females' readiness to mate.

I have made oft repeated attempts to observe the copulation process from the lateral aspect. Several devices, such as a trough made of cover glasses and a slide, in which the specimens were placed, yielded only failures. The males do not always attach to the same side of the female's carapace. In case the male attaches himself to the left side of the female's carapace, the spermatozoa are ejected near that side of the respiratory chamber and pass to the left of her abdomen to the brood pouch, seemingly being driven dorsad by the action of her last pair of appendages. In one such case observed the ephippial egg was developed in the right ovary. In order to enter the oviduct the spermatozoa would have to pass across the brood pouch to the right side. The sperm cells are immotile. Though I have not yet been able to see them after they have been trans-

ferred to the brood pouch, I am convinced that they are retained in it and that fertilization takes place after the egg is laid.

VII. General Discussion.

1. What relation does the presence of the males bear to the origin and prolongation of the duration of the sexual state in the females?

One gets the impression from the literature that the ephippial egg and ephippium are completely developed only if fecundation occurs. Perhaps enough data have already been given in the above discussion of the observations on pairing, of the effect of the presence of males in prolonging the duration of the sexual state of the females to convince any one that it is nil, though more evidence is at hand. I wish to add here that I have observed some hundreds of instances of embryo females developing through the stage at which they would become sexual, if at all, which never evinced the slightest evidence of sexuality. In a great many instances these were in the same containers with sister females which attained a high degree of sexuality. The males used in some of these experiments were related to the females, but in most instances they were not. It is evident that the ephippial egg and the ephippium are both developed to within one or two hours of the time of extrusion of the egg into the brood pouch, in complete independence of the male, the only definite correlations of these processes with the presence of males to be noted, being that the egg is usually not laid in the absence of fecundation, the exceptions being, as I should judge, about 1 per cent. to 5 per cent. I have observed about half a dozen instances.

2. In the same environment are individuals which pass through the sexual phase any more likely to give rise to males and sexual females than are individuals which are parthenogenetic from the beginning of their reproductive period?

Since the twenty-fourth female in isolation experiment 3 gave rise to pure broods of males, pure broods of sexual females, pure broods of asexual females, to mixed broods of males and females, and to pure broods of females of diverse sexuality, thus compassing the whole range of possibility as to kinds of offspring, she was presumably normal, hence I used some of her sexual

offspring with which to test this question. I selected 12 of the sexual females of broods 12 and 13. Each of these 12, after having produced one empty ephippium at the age of 11 days, became asexual. Two of the 12 died. In two other cases the first brood died too early for identification, but the first determinable broods of the 10 that lived were as follows:

1.	6	females,	1	sexual,	3	asexual,	and	2	undetermined.	
2.	7	"	2	"	3	"	"	2	"	
3.	3	"	0	"	2	"	"	2	"	
4.	3	"	0	"	3	"				
5.	2	"	0	"	2	"				
6.	10	"	4	"	6	"				
7.	6	"	1	"	5	"				
8.	3	"	1	"	2	"				
9.	12	"	1	"	11	"				
10.	6	males								

Thus in these first broods of the 10 sexual mothers only about 22 per cent. of the females are sexual, and about 12 per cent. of the offspring is male. All of the subsequent broods of these 10 mothers were also saved and identified. It was found that the percentage of males was slightly over 8 per cent., a decrease from that shown in the broods immediately following the ephippia. This can be of no special significance however since the percentage in that case is very much lower than one usually finds even in the offspring of asexual mothers, as noted in other experiments. As we have already seen, somewhat over 40 per cent. of the offspring, totaling over 1,700, of the 45 females concerned in isolation experiment number 2, is male. About 33 per cent. of the broods of these 10 sexual mothers are mixed as to sexuality, which is about normal. It will be recalled also that the percentage of males and sexual females in the sexual line of offspring from the stem mothers, (see isolation experiment 3) is actually slightly less than in the asexual line. It thus seems certain that the production of ephippial eggs at the beginning of the reproductive period, whether they are fertilized or not, has no influence on the subsequent offspring.

3. Is the age of the mother correlated in any way with the kind of offspring?

The twenty-fourth female in isolation experiment 2, gave as

her first two broods, pure male offspring. These were followed by three pure broods of females, and then a brood of 15 males. The seventh brood was a mixed brood. Her last 7 broods, totaling 66, were all females. The sexual and asexual females in these last broods occured in about equal number, namely, 30 sexual, and 36 asexual. Numberless such instances might be cited to show that males often appear in the first broods. The summary of the data in isolation experiment 2 shows that the first broods were pure male in 16 instances, pure female in 26 instances, mixed in 2 instances, and undetermined in 1; while the last broods were pure male in 11 instances, pure female in 28, and mixed in 6. The tabulated results in isolation experiment 3 are also in accord with the data just given and are, I believe, quite conclusive on this point. It will be noted that there is no marked increase or decrease in the number of males, sexual females, or of asexual females, from the first of the sixth broods, inclusive, in the descendants of the 24 stem mothers, for the three generations, in either the sexual or asexual lines. When the first broods of newly collected old and young females are compared no difference in kind is noted, as shown in experiment 5. When these same individuals are kept in small containers in the laboratory, no matter if the food supply is abundant, if the water is not changed often, sexual forms appear in the offspring of young and old alike, and in like proportions. These facts point to the conclusion that there is no correlation between the age of the mother and the kind of offspring.

4. Do mixed broods indicate a transitional stage from male producing to female producing and vice versa?

The summary of isolation experiment 2 shows 9 instances of the production of mixed broods which are preceded and followed by broods of the same sex, while there are but four cases in which mixed broods are preceded and followed by broods of different sex. There are in this same experiment two cases in which the first brood was a mixed brood, and 6 cases in which the last brood was mixed. Quite often one mixed brood follows another. I have found no evidence in favor of the view suggested by this question, the occurrence of mixed broods in these experiments being entirely capricious.

5. What relation has sexuality to the duration and senescence of the laboratory cultures?

The fact that my cultures which have produced sexual females and males have always passed sooner or later into a more or less non-productive phase and did not die out provided that conditions were not so severe as to terminate them, is well deserving of consideration, in view of the fact that one so often reads in Daphnian literature of cultures becoming sexual and being thereby terminated. During the five years I have worked on *Simocephalus* I have run scores of cultures and I have never had an instance of a pure sexual culture except those set up by selecting males and sexual females from other cultures. At the most such cultures remain purely sexual only a few days, when with the passing of some of the sexual females into the asexual phase, broods invariably appear containing asexual females. I have no record of a large pure brood of sexual females, and no record of a female which has consistently produced pure sexual small broods. Broods containing sexual females may be produced by any female no matter what her pedigree has been. Naturally if a large percentage of the females in a culture are passing through the sexual phase, so much of their immediate productivity is sacrificed, but since the number of ephippial eggs produced rarely exceeds 4, and is usually only 1 or 2, and always being produced within the first 10 to 25 days of their lives, every sexual female devotes the most of her life to asexual reproduction. Old cultures, in which there has been much accumulation of excreta, run down, the size of the broods decreases to from 1 to 5, many individuals die before maturity, and the culture dies out finally; but so far as I am able to see, this neither causes nor results from the production of ephippial eggs.

It is of interest to consider what happens in laboratory cultures which are set up and allowed to run their natural course unhindered. With each female producing a brood of from 3 to 35 every 40 or 50 hours, as is always the case in a culture containing an abundance of fresh green algæ and no accumulation of excreta, any culture, even though started with a single individual, will become overstocked in a few weeks, and the increase in numbers has to stop, the development of eggs in the ovaries and the

growth of the embryos become retarded, the culture remains nearly at a standstill for some weeks, and if the food supply is exhausted will finally die out, though it has been my experience that they usually linger on indefinitely. By the time the culture has become overstocked ephippial eggs have appeared in considerable numbers, and after having passed the state of equilibrium, when the rate of reproduction has rather abruptly ceased, for some weeks just about but not quite equaling the death rate, the accumulation of ephippial eggs may be taken as proof that the culture has passed through a sexual phase, which in turn is likely to be erroneously considered the cause of the decline of the culture. And now with reproduction at its lowest ebb, and all of the sexual females having passed into the asexual state, as they all do, the observer is naturally likely to believe that the culture has passed into the asexual phase, since all of the sexual members of the culture have yet to live the asexual phase of their lives. Thus there will be an indefinite period when nearly every member in the culture will be asexual. It must be remembered that at this critical period, assuming the ordinary proportion of males, reproduction must be cut down to somewhat less than two during the life time, which is a few weeks or months, of each female. Considering that 25 per cent. of the females are sexual (see table, isolation experiment 3), and that each sexual female is actually carrying an ephippium one-fifth of her life time, even assuming that to be but 14 days it is obvious that only 5 per cent. of the females will be carrying ephippia at any given time. I believe the life time in a large culture is in reality much longer than I have assumed, and 25 per cent. far too great, and that it is safe to say that in many instances the ratio of females actually carrying ephippia at a given time is as low as 2 per cent. If it should happen that these few should cast their ephippia at the same time, for a day or so thereafter one could find a sexual individual only by a tedious hunt with the microscope.

On the other hand, when reproduction proceeds apace, starting with 10 females let us say, each producing only 6 broods, with an average of 1 and $\frac{8}{10}$ sexual females to a brood, (as was the case in isolation experiment 3), we should have 78 ephippial

females in the first generation ($10 \times 6 \times 1.3 = 78$); and supposing there were in all only 5 females to a brood, (see table of same experiment), we should have 300 females to mother the F_2 generation, which should yield 23,400 ephippial females, out of a total of 90,000. Of course in practice, laboratory cultures are overstocked long before all of the second generation has appeared, and in fact reproduction has practically ceased by the time 100 to 200 ephippia have appeared. The accumulation of the ephippia makes the culture seem to be in the sexual phase. The variation in the number of kinds of offspring produced may depend to some extent upon the extreme prolificacy and consequent mortality in laboratory cultures, since the assumption of ever so slight a difference in the susceptibility of embryos to the great variety of adverse conditions met with in laboratory cultures, would be sufficient to account for much of the seeming variation. One may sometimes find several hundred ephippia in a laboratory culture in which there are very few males. In one such instance I collected 200 ephippia, only 2 of them containing eggs. Four explanations are possible: they may have hatched, which is very unlikely, there may have been too few males to fertilize them, mating may have been inhibited by some unfavorable cultural condition, or, there may have been abnormal pairing such as I have described in the section on pairing, in which case the spermatozoa not being retained, the eggs would degenerate. I have had a few small cultures in which for a short time males were very numerous and sexual females almost absent, but such instances are rare.

The following is typical of my experience with *Simocephalus*. I isolated a female on July 1, 1913, and kept her in lake water from lake Michigan. This was changed daily. She had nothing to eat except what she could get from the lake water. This seemed barely sufficient since reproduction proceeded very slowly. On August 1 I placed 6 of her descendants in a rich algæ culture. Here they multiplied very rapidly. By August 13 there were 50 ephippial eggs floating on the surface, and males were also present in the offspring. This culture continued to produce asexual forms for some months, when the algæ gradually died out individuals in the culture became less numerous, reproduction

having practically ceased. Another culture was established from this by selecting a few specimens at random and giving them a fresh supply of algæ. It ran through a similar period of great productivity, about the same number of sexual forms occurring, and declined as in the first instance. A third fresh culture was stocked by a single specimen from the last. It likewise gave rise to a great profusion of Daphnians, sexual forms appearing in considerable numbers. Unfortunately I did not keep a record of the time it took in the last two instances for the sexual forms to appear; but, as shown above, the first culture, started August 1, produced 50 ephippial eggs by August 13, and that in the presence of an enormous quantity of green algæ, the container being a three gallon jar which had previously been used for *Paramecia*. This *Paramecium* culture had been started with boiled wheat as the source of nutrition and had been permitted to stand for nearly a year. The *Paramecia* had disappeared and green algæ had developed in immense quantities on the side of the jar away from the light. Dearth of food could not have been a factor in inducing sexuality in this culture. I have already shown in isolation experiment 2 that sexuality comes on in spite of an abundance of food, in small vessels containing only a single individual female. The study of general cultures leads to conclusions which are in agreement with results obtained by a study of isolated females, namely, that onset of sexuality is independent of food shortage, and suggests that it is related to accumulations of certain excretions, which become critical in their effect upon the kind of offspring surprisingly early in *Simocephalus* cultures which are allowed to run their natural course unhindered. Production of ephippial eggs reaches its highest level about the time a general culture is over stocked and the rate of reproduction has begun to decrease, passing to lower levels as the food supply decreases, as is readily demonstrable if one will but take the trouble to remove them as they appear in such a culture, recording the daily output.

Of all the females isolated for study and kept in small containers in the laboratory, including several hundred selected at random and as many more whose pedigree was known for several generations, also 60 stem mothers and all of their female offspring that

lived, for three generations, the only instances in which both males and females did not appear in some of the broods were those in which they were very few, *i.e.*, in which the isolated females died early. The same was true of sexual females. They always appeared in some of the broods unless they were too few. Pure broods of sexual females were very rare and they have never predominated in any of my cultures no matter how severe the conditions were. Though I have not carried out a definite experiment to determine how many generations a culture may be carried through the sexual females alone (except in isolation experiment 3, where distinct lines were carried to the third generation) I have not the slightest reason to suppose that they may not be carried on indefinitely, since each female, whether sexual or asexual, gives rise to both sexual and asexual females, provided only that the food supply and other cultural conditions have proper attention. I am convinced that much of the confusion in the literature on the relation of sexuality to the senescence of cultures is due simply to the extreme prolificacy of the Daphnians and the complex phenomena resulting therefrom. Sexual phase and asexual phase may well be applied to individuals, and asexual phase to cultures, but in *Simocephalus vetulus* cultures never become wholly sexual, only partially so, since in the most sexual of cultures the offspring of a given female are always mostly asexual, and the sexual females pass most of their lives in the asexual state. Finally let us recall that at the extremely modest rate of production of ephippial eggs assumed in our calculations above, that starting with 10 females we should have 23,400 ephippia at the end of the second generation, whereas, as a matter of fact the most sexual of cultures of *Simocephalus vetulus* produces only a few during a course of many months. Hence it is folly to consider the onset of sexuality causally related to senescence of cultures in this species.

6. Does *Simocephalus vetulus* depend upon external factors to call into expression maleness, sexuality in the females, and parthenogenesis?

With respect to this point the results of Grosvenor and Smith (1913), Banta (1914), and Agar (1914b) are particularly instructive. They succeeded in completely inhibiting sexual forms in

several genera of Cladocerans for an indefinite number of generations. Their conclusions are more fully discussed under the head of literature review.

In my earlier experiments there are numerous instances of the production of all three kinds of individuals, namely, males, sexual females and asexual females, by the same mother. Less common is the production of only two kinds of offspring during the life time of a female. In these experiments I have no record of a female producing only one kind of offspring except those whose progeny are very limited because of the early death of the mother, and in recent experiments, those which produce only asexual females, this last being of much importance. Rarest of all yet quite often met with is the production of all three kinds of offspring in the same brood. Female number 45 of isolation experiment 2 has one such brood containing only three embryos. Broods of this type are usually larger. The asexual females have nearly always outnumbered the sexual females and males in mixed broods, as they usually do in the sum of the broods of a given female.

Similar proportions of the kinds of offspring may be secured under a great variety of kinds of environment. In the most highly sexual cultures many pure asexual females are always present, and the sexual females remain so only for a brief period in early life. While these facts do not prove that the environment has nothing to do with the ratio of kinds of offspring, they would seem to indicate that it is not the determinative factor in the nexus of causes that are responsible for the expression of the species in all of its forms, regardless of internal factors.

VIII. Summary.

I. *Life History.*

1. The offspring of any female of *Simocephalus vetulus* in the asexual phase may consist of any one or all three of the following kinds of individuals:

(*a*) Sexual females, which produce a series of from one to seldom more than six ephippial eggs early in life, then become parthenogenetic and so remain, being then indistinguishable from other parthenogenetic females. The final ephippium is

often only partially developed, showing the sexual capacity to be gradually lost.

(*b*) Parthenogenetic females, which display no tendency to produce ephippial eggs.

(*c*) Males.

The kinds of offspring occur in no definite order, but their character is probably determined at birth, and not by subsequent conditions. (See summaries of isolation experiments 2 and 3.) Eggs which will develop into males, into highly sexually females, and parthenogenetic females, often arise in the same female and not infrequently at the same time, either early or late in her reproductive period, whether or not she has passed through the sexual state herself.

2. The sequence of the generations is very indefinite:

(*a*) The stem mother is functionally like the females produced parthenogenetically, except that she probably never gives rise to ephippial eggs. (However see Sharfenberg, 1911, p. 24.) There is not even an approximately definite number of generations in the cycle from one stem mother to another. It may be one or many.

(*b*) The remoteness of the generations from the stem mother bears no definite relation to the percentage of males produced, the ratio of sexual to parthenogenetic females, or to the duration of the sexual state when present.

(*c*) The sexual state is probably determined in the ovary of the preceding generation. There are almost certainly predisposing factors in the environment but it is not certainly known what they are. Food or lack of food does not offer a sufficient explanation. Sexual females and males tend to arise at the same time, presumably in response to the same environmental complex.

(*d*) Cultures are indefinitely viable parthenogenetically. The species will express itself in all of its forms under a great variety of conditions. Under certain conditions sexual forms are completely inhibited. Parthenogenesis cannot be completely inhibited in cultures or even in individual females. Thus cultures of *Simocephalus vetulus* can never be terminated merely because of the onset of sexuality.

(*e*) The production of mixed broods is not to be interpreted

as evidence that the female producing them is undergoing transition from male producing to female producing or vice versa.

3. The production of ephippia and ephippial eggs are related but not causally, both being dependent upon common internal factors. The introduction of males into a culture does not induce the production of ephippial eggs, nor does their presence have any relation to the prolongation of the sexual state when it has once appeared.

II. *Breeding Habits.*

Sexual attraction is limited not only to sexual females in the sexual state, but is confined to a limited period of a few hours before the ephippial egg is laid. It seems to be due to some kind of substance omitted by the female and borne by her exhalant respiratory current, where it is detected through a chemical sense by the male. Fertilization seems to take place in the brood pouch after the egg is laid. The presence of the spermatozoa in the brood chamber is probably the stimulus for its extrusion. In the absence of fertilization the ephippial eggs are usually resorbed in the ovary, or, if laid they undergo degeneration in the ephippium within one or two days.

III. *Theoretical.*

The immediate significance of the ephippial egg is as a stage in the life cycle resistant to adverse conditions, and not in the stem mother hatching from it, since her offspring are in no way unique. A more remote but more fundamental significance, held in common of course, with all fertilized eggs and zygotes, is that it provides for the permanent lability of the species through amphimixis. In view of the great prolificacy of the species in regard to all of its forms, and the almost universally concomitant occurrence of males and sexual females, the development of only 1 per cent. of the ephippial eggs would be quite sufficient to secure to the species all of the benefits to be derived from their two functions. It seems probable that there is a very great inherent variability in the capacity for development of the ephippial eggs in a state of nature, and the lack of uniformity of results obtained by the various investigators in their attempts to shorten the latent period may be explainable on that basis.

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MITOTIC DIVISION OF BINUCLEATE CELLS.

ETHEL BROWNE HARVEY.

Attention has been called to the presence of binucleate cells in the follicular epithelium of insects by several observers (Korschelt, '87, Preusse, '95, De Bruyne, '97, Gross, 01, et al.). In *Notonecta*, binucleate cells are characteristic of the follicular epithelium and also of the tissues lining the sperm duct and investing the spermatogonial cysts. These binucleate cells undergo normal mitotic division, both nuclei being involved. As a usual rule, both nuclei pass through the stages of division synchronously, though rarely one finds one nucleus in advance of the other. (Fig. 11, upper cell.)

In the resting stage (Fig. 1), each nucleus is exactly similar to the single nucleus of a spermatogonial or oögonial cell, a conspicuous karyosphere being present (Browne, '13). In the prophase, the two nuclei remain distinct and thick chromatin strands are present in each nucleus (Fig. 2, upper cell, Fig. 3). When the nuclear wall breaks down in the late prophase (Fig. 4), the two nuclei may undergo a certain amount of fusion, or they may remain entirely distinct and this is true also of the metaphase. In some cases, two distinct plates may be seen in polar view (Fig. 5) or two distinct spindles in side view (Fig. 6); in fact, the two spindles may be so independent as to lie in different planes, so that one is seen in side view and the other in polar view (Fig, 7). In other cases, the two spindles become so intimately combined as to appear as one giant spindle. In polar view a perfect equatorial plate is seen, but there are *twice as many chromosomes present as the diploid number for the species.* Each chromosome of the spermatogonial or oögonial metaphase is represented by two exactly similar chromosomes in these binuclear plates, as may be seen by comparing Fig. 8 from an oögonial cell of *Notonecta indica*, with Fig. 2 (lower cell) from a follicle cell of the same species. In the oögonial cell, there are 6 large, 4 small and 16 intermediate sized chromosomes, in

the binucleate follicle cell there are 12 large, 8 small and 32 intermediate ones. Similar double chromosome plates have been found in the follicle cells of other insects, *e.g.*, *Anasa* (Wilson, '06) and are probably likewise due to the division of a binucleate cell.

In the anaphase (Fig. 9), the two sets of chromosomes cannot be distinguished, and the two spindles appear always to be fused. It is difficult to tell exactly what happens in the telophase. It would seem that a membrane formed around the group of chromosomes at each pole, (Fig. 10), each daughter nucleus becoming elongated and that it later separated off into two nuclei (Figs. 11 and 12). It seems entirely possible that in such a separation into two nuclei, which would resemble a nuclear amitosis, the chromosome content might be so distributed that each nucleus would maintain the chromosome constitution characteristic of the species. It is possible that the separation into two nuclei occurs only in those cases where the original sister nuclei did not become entirely fused and that when they are fused, each daughter nucleus remains as an abnormally large nucleus, really double in nature. It is evident, however, from the series of figures that two binucleate cells may arise by the mitotic division of a binucleate cell.

Although the binucleate cells of the follicular epithelium have been believed by some observers (Preusse, '95, De Bruyne, '97, Gross, '01), to arise by an amitotic division, I have found no evidence of such an origin, and would believe it probable that they arise by the failure of cell division after a nuclear division by mitosis. Macklin ('16) has described a process of amitotic nuclear division in his study of binucleate cells in living tissue, but the figures he gives might also be interpreted as the last stages in the mitotic division of a binucleate cell as described above. If binucleate cells do arise as Macklin believes and his interpretation of them as potentially mononuclear, is correct, there must be two types of binucleate cells. In one type, each of the two nuclei has a complete set of chromosomes, as described above. In the other type, the two nuclei taken together have a complete set of chromosomes. Macklin was, however, unfortunately unable to verify his assumption as to the chromosome

constitution of his binucleate cells. Binucleate cells, arising by the suppression of cell division after nuclear division by mitosis have also been produced experimentally in the cleavage cells of *Crepidula* (Conklin, '12). The two nuclei may divide mitotically and entirely independently although synchronously; in these cases, however, two mononucleate cells are usually produced from the original binucleate cell.

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EXPLANATION OF PLATE I.

Fig. 1 from the sperm duct of *Notonecta shooterii;* Fig. 8 from oögonial cell of *N. indica*, other figures from follicle cells of *N. indica*.

FIG. 1. Binucleate cells from sperm duct of *N. shooterii*, resting stage.

FIG. 2. Upper cell, prophase. Lower cell, metaphase showing a perfect double chromosome group, 52 chromosomes.

FIG. 3. Prophase.

FIG. 4. Late prophase.

FIG. 5. Metaphase, polar view, two plates separate.

FIG. 6. Metaphase, side view, two plates separate.

FIG. 7. Metaphase, two spindles in different planes.

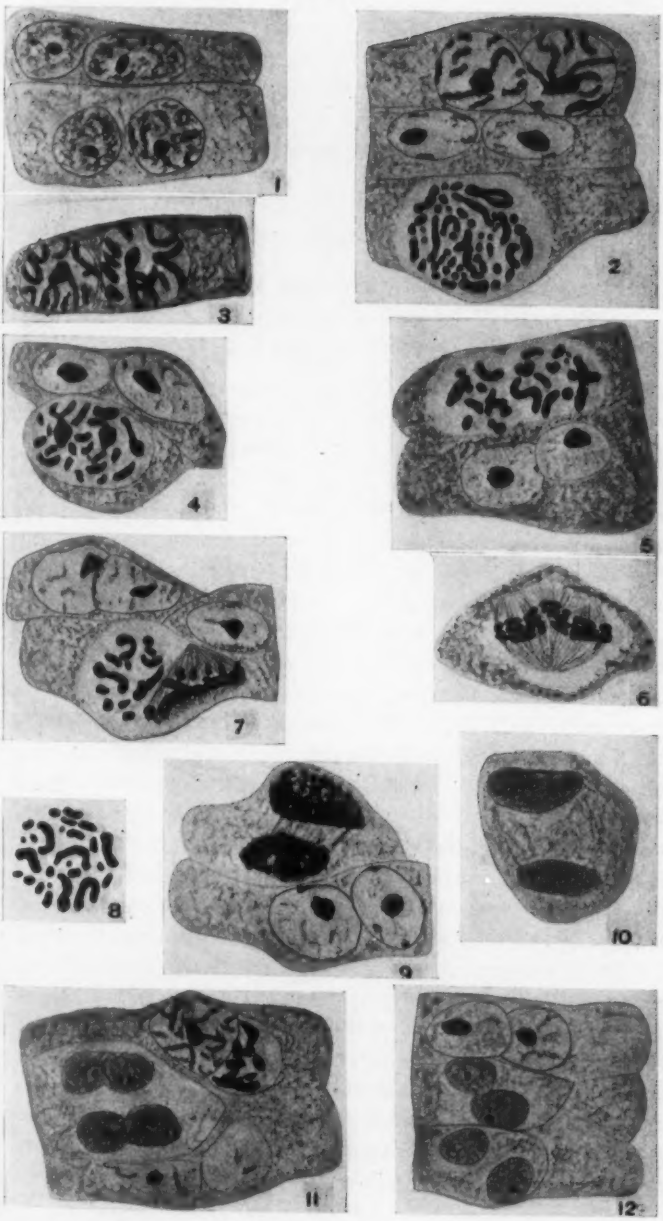
FIG. 8. Metaphase of oögonial cell of *N. indica*, showing 26 chromosomes.

FIG. 9. Anaphase.

FIG. 10. Telophase.

FIG. 11. Lower cell, telophase, sister plates dividing into two. Upper cell one nucleus in prophase, one in metaphase.

FIG. 12. Late telophase showing two daughter binucleate cells.



ETHEL BROWNE HARVEY.

THE AXIAL GRADIENTS IN HYDROZOA.

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II. SUSCEPTIBILITY IN RELATION TO PHYSIOLOGICAL AXES, REGIONS OF COLONY AND STAGES OF DEVELOPMENT IN CERTAIN HYDROIDS.

The present paper is a study of the susceptibility relations in certain species of colonial hydroids and includes data bearing upon the following points: axial susceptibility in single hydranths, medusa buds and stems; susceptibility in relation to stage of development, physiological age and motility; regional differences in susceptibility in colonies and their relation to growth form and general physiological condition; susceptibility in acclimation. These data have been accumulated during some five years past, in part at Woods Hole and in part at Friday Harbor, Wash. The forms chiefly studied are *Pennaria tiarella* and *Tubularia crocea* at Woods Hole and *Bougainvillea* sp., *Obelia geniculata* and *O. borealis* at Friday Harbor. Some work was also done with a third unidentified species of *Obelia* and with another campanularian, *Gonothyria clarkii*, also at Friday Harbor. I am indebted to Professor Trevor Kincaid and to Professor C. C. Nutting for identification of certain of the Friday Harbor species.

For the direct determination of susceptibility to slowly lethal concentrations of agents the method is the same as that used for *Hydra* (Child and Hyman, '19) and many other forms, viz., the determination of the time of death as indicated by the disintegration or cytolysis of the protoplasm in KNC, HCl, various anesthetics, neutral red, methylene blue, or in some cases by the time before decoloration in KNC or other neutral or alkaline agents after staining with neutral red. This decoloration occurs only when the protoplasmic structure disintegrates: the first stage is a change in color of the neutral red to yellow as the sea water or the alkaline agent penetrates all parts of the protoplasm,

and following this the yellow color gradually disappears in most cases, the dye being no longer held by the dead protoplasm. The vital dyes, neutral red and methylene blue have been used both as agents for staining intra vitam and for determining differences in susceptibility. In the investigation of acclimation in lower concentrations of KNC, HCl, ethyl urethane, $MgSO_4$, neutral red and methylene blue, it was found that different hydranths or regions of the colony show differences in capacity for acclimation.

The question of the value of susceptibility as a criterion of certain quantitative aspects of physiological condition has been discussed so many times in earlier papers (see, for example, Child, '19*b*, and the references there given), that no further consideration of the matter is necessary here. It need only be said that a rapidly increasing volume of evidence along many different lines indicates that in general susceptibility to higher concentrations or intensities of at least many agents, as indicated by death or inhibition, as well as capacity for acclimation to, or recovery from the action of lower concentrations or intensities, vary in general, though not necessarily proportionally to the rate at which fundamental physico-chemical processes are going on in protoplasm.

Since adequate presentation of the experimental protocols on susceptibility would require much space, only the general results are stated, but for each statement made there is an extensive experimental basis. Moreover, the experiments demand no difficult technique and can easily be repeated, the chief precaution to be observed being the distinction between freshly collected material in good condition and that which has been kept in the laboratory. With some species even a few hours under laboratory conditions are sufficient to alter the susceptibility relations of different parts to a considerable degree.

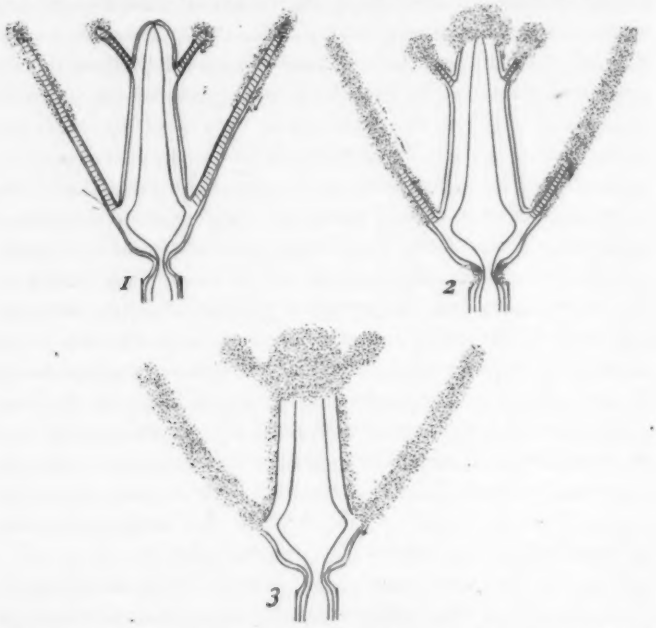
The Death Gradients in Hydranths.

The observations on hydranths have been made repeatedly, both on the different hydranths of single colonies and different colonies of the species mentioned above, and the progress of disintegration has been observed in hundreds of cases. The

hydranth of *Pennaria tiarella* will serve as an example of the course of disintegration in the hydranth of a tubularian hydroid. In full grown hydranths in KNC *m*/800 to *m*/400 approximately, in neutral red, or in KNC after staining with neutral red to make the death changes more clearly visible, disintegration begins in the ectoderm at the tips of the proximal tentacles and progresses basipetally, usually at about the same rate in each tentacle (Fig. 1). Some time later, usually when disintegration has progressed over one to two thirds the length of the proximal tentacles, it begins in the ectoderm at the tips of the distal tentacles and progresses basipetally in these also, reaching the bases of both series of tentacles at about the same time. At, or often somewhat before this time, ectodermal disintegration begins at the tip of the manubrium and progresses basipetally over the manubrium and body of the hydranth (Figs. 2 and 3). The short stalk below the hydranth differs in relative susceptibility in different cases, and this difference, as in the case of the 'stalk' of *Hydra* (Child and Hyman, '19) apparently depends on the occurrence or non-occurrence of contraction. In cyanide, particularly the higher concentrations, this stalk usually contracts and in such cases its ectoderm disintegrates at about the same time as that of the mouth region (Fig. 2), but where contraction does not occur, it disintegrates only after ectodermal disintegration of the body is completed (Fig. 3).

In general the entoderm disintegrates later than the ectoderm, except near the mouth region. Since the agent must pass through the ectoderm to reach the entoderm, this difference is perhaps not significant, though it may be noted that in neutral red the entoderm stains almost as rapidly as the ectoderm and may stain much more deeply than the latter before death occurs. In the proximal tentacles the solid column of entodermal cells shows a basipetal susceptibility gradient, like the ectoderm, disintegration beginning at the tip of the entodermal column when ectodermal disintegration has progressed half way more or less to the base (Fig. 1). In the distal tentacles the difference in susceptibility between ectoderm and entoderm is similar. In the mouth region the entoderm may disintegrate as early as, or sometimes earlier than the ectoderm, perhaps because the

agent enters through the mouth to some extent, though, so far as observed, the mouth remains closed until disintegration begins in that region. As regards the rest of the body and the stalk, entodermal disintegration is distinctly later than ectodermal and is usually without any very clearly marked gradient.



FIGS. 1–3. Diagrammatic optical sections of *Pennaria* hydranths to show the course of disintegration in lethal concentrations. Fig. 1. Ectoderm of proximal tentacles disintegrated half way to base; entoderm beginning at tips. Ectoderm of terminal knobs of distal tentacles disintegrated. Fig. 2. Ectoderm disintegrated to bases of both series of tentacles; entoderm of proximal tentacles two thirds disintegrated, of distal tentacles half disintegrated. Ectoderm of short stalk below hydranth disintegrating. Fig. 3. Tentacles, hypostome and apical one third of hydranth body completely disintegrated. Ectoderm of hydranth body disintegrated to bases of proximal tentacles. Ectoderm of stalk intact.

Here as in *Hydra* (Child and Hyman, '19, p. 193) the entoderm of the mouth region is apparently much more susceptible than that of other body regions, a difference which is perhaps associated with specially intense glandular activity in this region, of which there is some histological evidence.

The length of time between exposure to the agent and the beginning of disintegration and the rate of its progress may of course be varied widely by varying the concentration. The concentrations most used are those in which disintegration begins in one half to one hour after exposure and is completed in three to six hours.

The susceptibility relations in the well developed hydranth of *Tubularia crocea* and in *Bougainvillea*, except that in the latter there is only one series of tentacles, are similar to those in *Pennaria*. The campanularian hydranths examined also show essentially the same relations in fresh material.

The Death Gradients in Stems and Stolons.

Data on susceptibility of stems to chemical agents, as measured by disintegrative changes, are of course open, in all hydroids possessing a perisarc, to the possible objection that differences in permeability and thickness of the perisarc at different levels may constitute complicating, or perhaps the chief factors in determining the time of death. The thickness of the perisarc is least in the most recently developed, *i. e.*, in any particular axis, the most apical portions of the stem and its thickness increases in general basipetally.

As a matter of fact, however, the perisarc is apparently penetrated rather readily by many agents. It does not obstruct or retard greatly the entrance of vital dyes and permanganate penetrates it almost at once, even where it is thick. While the possible complicating effect of the perisarc must of course be admitted, various facts cited below indicate clearly enough that it is not responsible for all the differences in susceptibility observed in stems.

In general a stem is less susceptible than the hydranth it bears, and the susceptibility decreases basipetally in the stem, at least near the hydranth, in all agents used in lethal concentration. In concentrations of KNC, neutral red, etc., which do not kill within a few hours, the more apical portions of stems usually undergo resorption and retraction within the perisarc after more or less complete disintegration or resorption of the hydranths. These processes of resorption and retraction of the more apical

regions are themselves indications of a differential susceptibility in the stems.

The staining with neutral red may be apparently uniform over many millimeters of stem length, while considerable differences in susceptibility, as indicated by the progress of disintegration and decoloration may appear in the same region. As regards the permeability of the perisarc in different regions to KNC, nothing definite can be said, but the susceptibility gradients in KNC are similar to those in neutral red.

The differences in susceptibility are most marked near the apical ends of stems and stolons, where the differences in rate of growth are greatest within short distances. The growing tip of a stem or a stolon is its most susceptible region, and the susceptibility decreases rapidly from the tip basipetally within a length of several millimeters to a few centimeters, according to the species and the conditions of the stem. In the older regions of stems and stolons differences in susceptibility are much less marked, even over length of several centimeters, and irregularities frequently appear, as in the older portions of the thalli of algæ (Child, '16*a*, '16*b*), some regions dying earlier than regions above or later than regions below. In these older parts susceptibility is often the same in regions where differences in thickness of perisarc are considerable, or it may be different in regions covered by perisarc of equal thickness.

In general the apical region of a growing stem which is to develop into a hydranth is more susceptible than the apical region of a growing stolon of the same colony, but no constant differences have been observed between the older regions of stems and stolons. That these differences between apical ends of stems and stolons represent differences in physiological condition is indicated by the fact, to be more fully discussed in a later paper, that the apical ends of stems can be reversibly transformed into stolons by the action of low concentrations of inhibiting agents.

These data on susceptibility in stems and stolons are in agreement with the facts of observation in that they show the regions of greatest growth activity to be most susceptible to lethal concentrations, and the differences in susceptibility cannot be in-

terpreted wholly in terms of permeability or thickness of perisarc. According to the evidence, the susceptibility relations in the hydroids are very similar to those observed in the multiaxial thalli of algæ (Child, '16*a*, '16*b*, '17), and certain other resemblances will be pointed out below.

The Death Gradients in Developing Medusa Buds.

The elongated, naked medusa buds of *Pennaria* have constituted the chief material for these observations. In the earlier stages of the bud the disintegration of the ectoderm begins at the free, apical end and progresses basipetally (Fig. 4). In

Figs. 4–6. Stages of disintegration in Medusa buds of *Pennaria*. Fig. 4. Ectodermal disintegration beginning apically in very early bud. Fig. 5. Later bud stage with disintegration beginning on the tentacular outgrowths. Fig. 6. Later stage of disintegration.

later stages, where the tentacular outgrowths have begun to develop, these disintegrate first of all (Fig. 5), and the process then progresses basipetally over the body, as in earlier stages (Fig. 6). The entoderm disintegrates somewhat later than the ectoderm and shows, at least in earlier stages, a basipetal gradient. The apical region of the developing medusa bud of *Pennaria* represents of course the marginal region of the fully grown medusa, the subumbrellar region not being open to the exterior until an advanced stage of development is reached.

The earlier stages of the gonophores of *Tubularia* likewise show a basipetal susceptibility gradient in the ectoderm and a similar, less distinct gradient in the entoderm. The conditions in the fully developed, free swimming hydromedusae will be considered at another time.

Differences in Susceptibility with Stage of Development.

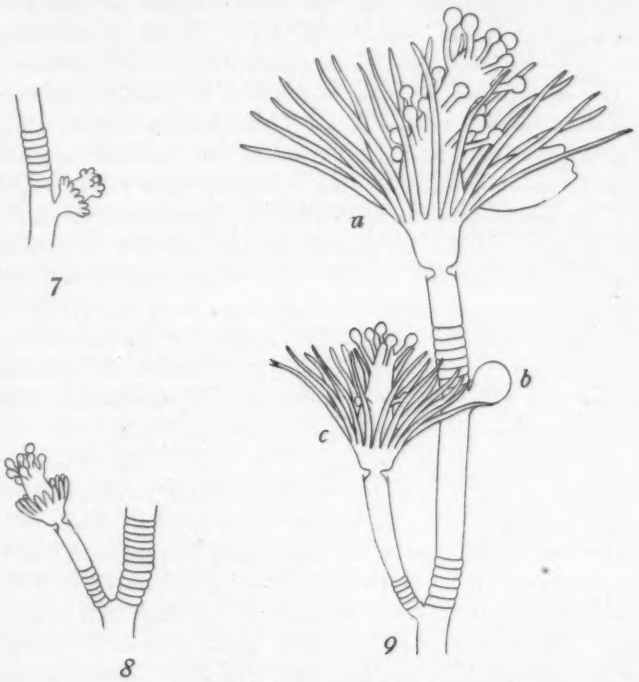
The appearance of motor activity during hydranth development affects susceptibility to some extent as in the case of *Hydra* (Child and Hyman, '19). In general motor activity increases susceptibility, as numerous experiments, not only with *Hydra* and hydroids, but with other forms have shown. The effect of motor activity on susceptibility in *Planaria* has long been used as an experiment in laboratory class work. The earlier stages of hydranth development are non-motile, the later stages motile, *e.g.*, in *Pennaria* the stage of Fig. 7 shows no motor activity, the stage of Fig. 8 is slightly motile and the stages *a* and *c* in Fig. 9 are highly motile. In spite of this difference the early stage of Fig. 7 is distinctly more susceptible than Fig. 9, *a*, but between the stages of Fig. 7, Fig. 8 and Fig. 9, *c* the differences in susceptibility are not very great. In stages after motility is fully developed the susceptibility shows in general a decrease with increasing size of the hydranths on a particular branch or in a particular region of the colony, except that in monopodial forms such as *Pennaria* and *Bougainvillea* certain peculiar conditions exist in the terminal hydranths of the colony and main branches (see p. 114 below).

The effect of motor activity on susceptibility is also evident in the susceptibility of the tentacles, as compared with that of the hydranth body in different developmental stages. In the early stages, *e.g.*, Fig. 7, the tentacles are non-motile and disintegrate at about the same time as the region of the body to which they are attached, but after they develop motility, they are the most susceptible portions of the hydranth, as noted above, and in the forms with two series the proximal tentacles are more susceptible than the distal. In *Pennaria* the proximal tentacles of the advanced stage, Fig. 9, *a*, begin to disintegrate at about the same time or a little later than those of the early stage, Fig. 7, but at this time the body of the early stage is disintegrated down to the level of the proximal tentacles, while the body of stage *a*, Fig. 9 does not begin to disintegrate until later, the actual times depending on concentration of the agent used.

In all other forms examinèd, *Tubularia*, *Bougainvillea*, the

campanularian, *Obelia*, the earlier stages are in general more susceptible than the later, though some differences depending on motor activity and on position in the colony (pp. 112–117) appear.

The earliest stages of bud development (Fig. 9, *a*, Fig. 10, *g*, are, with rare exceptions, less susceptible than naked early hydranths. Since these early stages are enclosed in a thin perisarc they are perhaps not directly comparable as regards susceptibility with later naked stages, but in *Obelia* they are in general less susceptible than later stages such as *b* and *f*, Fig. 10, which are also enclosed in perisarc. On the other hand, they are more susceptible than the stem regions from which they arise. These



FIGS. 7–9. *Pennaria tiarella*. Figs. 7 and 8. Early hydranth stages. Fig. 9. Apical region of a stem, showing monopodial form of branching. The hydranth *a* remains apical, the stem below it increases in length and the bud *b* becomes a lateral branch like *c*.

facts indicate that real physiological differences exist in the different bud stages. Probably the lower susceptibility of the earliest as compared with later stages means that they have not yet attained their maximum physiological activity as regions of growth. The period of early bud development is certainly a period of acceleration in physiological activity and so of increasing susceptibility like the earlier stages of embryonic development (Child, '15*a*, Chap. XV.). Later, as differentiation of the hydranth progresses, susceptibility again decreases.

The sensitiveness of these hydroids to laboratory conditions, as well as the close relation between susceptibility and physiological condition is indicated by the fact that colonies kept over night in standing water or even in aquaria with running water very frequently show, either little or no difference or a more or less complete reversal as regards the susceptibility of different stages of development to KNC, *i.e.*, those stages which were originally most susceptible are either no more susceptible or even less susceptible than other stages. A similar reversal appears when the animals are stained for several hours in neutral red and then killed in KNC. Reversals have also been observed in various algæ under the same conditions (Child, '16*b*, '17). In the hydroids, as in the algæ, it is evident that laboratory conditions or neutral red alter the physiological condition of the earlier, originally more susceptible developmental stages to a greater degree than that of the later stages, and that this alteration decreases the susceptibility of the earlier stages to KNC to such an extent that it is equal to or less than that of later stages. Since susceptibility to KNC shows a close relation to rate of oxidation, it is probable that laboratory conditions and neutral red decrease oxidation in some way and to a greater degree in earlier than in later stages. In other words, this effect of laboratory conditions is itself dependent upon the differences in susceptibility which exist in these organisms. Moreover, this effect and that of neutral red on the one hand, and that of KNC on the other are evidently not additive, as the effect of KNC and lack of oxygen appear to be (Child, '19*b*).

The susceptibility of the medusa bud, like that of the hydranth, decreases with advancing development in all stages preceding

the development of motor activity, except the very earliest. In *Pennaria* the earliest visible stages show no distinct difference in susceptibility from the hydranth body on which they arise, but somewhat later stages (Fig. 4) are slightly more susceptible than the hydranth body, though distinctly less susceptible than young hydranths of the stage of Fig. 7. Half grown buds are almost always slightly less susceptible than the hydranth body to which they are attached, and the still later stages are much less susceptible than the hydranth and are the least susceptible structures in the colony except the older parts of the stems.

In *Tubularia* the gonophores, which are modified medusæ, show a decrease in susceptibility from a certain early stage on, and the later stages are less susceptible than the hydranth from which they arise. In *Bougainvillea* also, where the medusæ arise singly from special buds instead of from the hydranth body, the differences in susceptibility with stage of development are the same.

The gonozoöid of the campanularian species examined is in general less susceptible than the fully developed hydranth, and even the earlier stages of gonozoöid development are less susceptible than early hydranth stages, though more susceptible than late hydranths, in spite of the presence of perisarc. The medusa buds on the blastostyle show differences in susceptibility with developmental stage similar to those observed in the medusa bud of *Pennaria*, the advanced stages being much less susceptible than corresponding hydranth stages.

The results with medusa buds of different species are then consistent. The medusa bud represents a reproductive process with a certain amount of dedifferentiation in the earliest stages, and in these stages some increase in susceptibility appears, as compared with the region from which the bud arises, but as differentiation progresses, the susceptibility again decreases. The changes are similar to those in the hydranth, except as the latter are masked by the development of motor activity in relatively early stages. The susceptibility of the medusa bud, however, is in general lower than that of corresponding stages of hydranth development, a fact which agrees well with the greater differentiation of the medusa. In short,

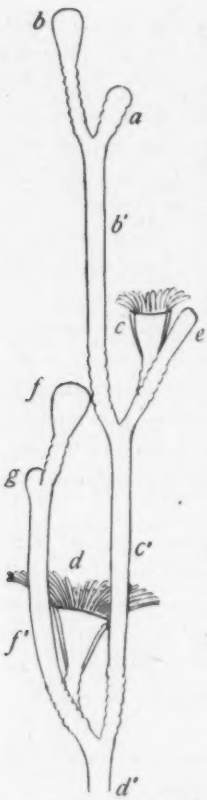


FIG. 10. Apical region of stem of *Obelia borealis* Nutting, to show sympodial method of branching: (*a*) Latest bud on main axis; arose as a lateral bud from the axis *bb'*. The axis *bb'* arose as a lateral bud from the axis *cc'*, and axis *cc'* as lateral bud from axis *dd'*. As the stem of hydranth *d* elongated a second bud appeared and produced the axis *ff'*, and from this axis a new axis *g* is just developing. The stem of hydranth *c* has also produced a second bud which has given rise to the axis *e*. In this way the lateral branches develop.

the susceptibility relations indicate that the medusa represents physiologically a more advanced stage of development than the fully developed hydranth and is therefore physiologically older and less susceptible.

COLONY GRADIENTS.

The hydroid colony as a whole represents a more or less orderly integration of the constituent units. The general growth form or arrangement of zoöids is more or less definite and characteristic for the species, and this definiteness of form and arrangement is particularly characteristic of those species in which a main stem and lateral branches, with perhaps secondary and tertiary branches can be distinguished. In these respects the hydroid colonies resemble multiaxial plants, and such plants are colonial forms in the same sense as the hydroids, *i.e.*, each growing tip in the plant, like each zoöid in the hydroid, represents at least the apical region of a physiological axis, an individual, with more or less complete development of other parts. Moreover, the two general types of relation between the different constituent axes which are characteristic of plants, viz., the monopodial and the sympodial forms of branching, are also found in the hydroids. In *Pennaria* and *Bougainvillea* and various other tubularian hydroids the colony is of the monopodial type, *i.e.*, new axes arise as lateral branches of a persistent main axis (Fig. 9). In *Obelia* and various campanularians examined the colony is sympodial in type. Here each new bud becomes the apical end of the apparent main axis, the former apical zoöid being dis-

placed and becoming the tip of a lateral branch (Fig. 10). In these forms what is apparently the main axis is actually made up of a portion of each successive axis formed. In the monopodial *Pennaria* the apical zoöid is, except for injuries or losses, the first zoöid developed and so the oldest in point of time of that stem or branch, while in *Obelia* the apical zoöid is the latest developed and the youngest in point of time, except for the new bud which is soon to take its place.

In both types, however, certain characteristic relations exist between main axes, real or apparent, and lateral branches. In *Pennaria* and the *Obelia* species examined, for example, lateral branches grow more slowly than the main axis, so that the colony developing without injury or crowding shows more or less the general form of a conifer. It has been experimentally determined that in plants of such form very definite physiological relations exist between main and lateral axes, and it has been possible to show for various algæ of these growth forms that the susceptibility relations are likewise very definite in character (Child, '16*a*, *b*, '17). The similarity of growth form in plants and hydroids indicates that the physiological relations between the constituent members of the complex must be in certain respects similar and therefore quite independent of the specific differences in plant and animal protoplasm, in other words quantitative in character.

The susceptibility of the hydranths of different regions of the whole colony or of particular stems or branches shows certain characteristic differences which are similar to those found in the algæ and associated in a definite way with the growth relations of the constituent axes. In a well developed colony of *Pennaria* all primary branches, except those latest formed just below the apical end of the main axis, terminate in large hydranths of about the same developmental stage, so far as appearance is concerned, though of course, where losses and regenerations have not occurred, the apical hydranth of the main axis is the oldest in point of time, that of the most basal branch the next oldest and that of each successive branch successively younger. In fresh material, however, the susceptibility of these full grown terminal hydranths decreases in general basipetally in the

colony, *i.e.*, the terminal hydranth of the main axis, although the oldest of all in point of time, and the terminal hydranths of the more apical branches, which are, as regards time, the youngest terminal hydranths, are on the average more susceptible than terminal hydranths of more basal branches. For example, characteristic cases show 60–80 per cent. of the fully developed terminal hydranths of the apical half of a colony disintegrating at a time when 60–80 per cent. of the terminal hydranths of the basal half are still intact. In any well developed primary branch bearing secondary branches, the susceptibility differences in the terminal hydranths of the secondary branches are in general similar to those in the primary terminal hydranths. The terminal hydranth of the primary branch and the hydranths of the more apical secondary branches are on the average more susceptible than those of more basal secondary branches. In short, the colony as a whole and each larger branch shows definite and orderly susceptibility relations among its hydranths, and these relations are similar to those observed in algae with similar growth form. *Bougainvillea* shows similar susceptibility relations, but as might be expected from the less regular form of colony they are less distinct and uniform than in *Pennaria*. It is perhaps necessary to emphasize the point that these are general and average differences and are best determined by comparing percentages of terminal hydranths in process of disintegration at a given time in different regions of the colony, rather than by comparing individual hydranths. The differences are not as great as the differences with stage of development and are clearly brought out only by some statistical method. Even the apical hydranth of the colony does not always disintegrate earlier than any other fully developed hydranth, but it does do so in a large proportion of the cases in fresh material and the relative susceptibility of the other terminal hydranths is likewise not absolutely uniform. In fact this relation in the hydroid is much less distinct and uniform than in many of the algæ.

It is a fact of some interest that in *Pennaria* and *Bougainvillea* the apical hydranth of the colony is the most susceptible or among the most susceptible of the fully developed hydranths, although the oldest in point of time, and that the terminal

hydranths of branches differ in susceptibility as noted above, according to their position in the colony. These differences in physiological condition indicate that for some reason the terminal hydranths of the colony and many branches undergo physiological senescence less rapidly than others. In this connection it may be pointed out that the terminal hydranth is more closely associated than any other with the process of hydranth reproduction, since the new hydranth buds arise a short distance below it (Fig. 9). Moreover, growth and the development of new buds occurs most rapidly in the apical region of the main axis of the colony, and the rate of growth and budding decreases basipetally in the colony as a whole and in each branch. Apparently, as in those plants with similar growth form, there is a relation of dominance and subordination in the colony and in each branch, the apical region being dominant and inhibiting growth and reproduction in other axes to a greater or less degree.

Since new buds arise a short distance below the apical hydranth of any axis (Fig. 9), they probably produce some effect upon the hydranth, either in the way of reorganization or through the demand for nutrition which they create. The nutrition for the bud must be derived largely from the reserves or the newly digested food of the apical hydranth, consequently the nutritive condition of this hydranth may be altered to some degree in the direction of starvation, but when the new hydranth becomes able to obtain food for itself the apical hydranth may recover its former nutritive condition. The effect of starvation in producing regression to a physiologically younger condition in the lower invertebrates has been clearly demonstrated (Child, '14, '15*a*, Chap. VII, '19*a*) and it seems probable that alternating changes of this sort associated with the development of new buds are concerned in the retardation of senescence in the apical hydranths. In a monopodial colony such as *Pennaria* the hydranth in which such changes occur most frequently, *i.e.*, the apical hydranth of the colony, will, according to this hypothesis, undergo senescence least rapidly, and the relative rate of senescence in the apical hydranth of any axis will vary in general inversely as rate of growth and frequency of reproduction in that axis. Consequently, since rate of growth and frequency

of reproduction decrease basally in the colony and its branches, the more basal hydranths and regions undergo senescence more rapidly.

The whole complex of physiological relations in the colony represented by the differences in rate of growth, reproduction and susceptibility in different regions results from the order of development. The colony begins as a single axis terminated by a hydranth. When the stem attains a certain length a bud appears at a certain distance below the terminal hydranth and is probably inhibited to some extent by it. As growth of the main stem continues, a second bud arises above the first and now the earlier bud or hydranth is subjected to the inhibiting action of two apical regions, and so on as other buds develop. The same relations arise in each branch as it develops. It follows that in any axial complex the degree of inhibition will increase basipetally.

In *Obelia* and other sympodial forms the situation differs in one respect from that in the monopodial *Pennaria*. The apical hydranth of the colony and of each branch in *Obelia* is the latest developed, the youngest both in point of time and physiologically of the axial complex which it terminates. In well developed colonies of *Obelia*—colonies of *O. borealis* 10–14 cm. in length and very symmetrical in general form are common at Friday Harbor—a general apico-basal gradation in susceptibility of these young apical hydranths or buds of different primary branches appears, those of more apical branches of the colony being somewhat more susceptible than those of more basal branches, like the terminal hydranths of *Pennaria*. Similar differences appear to some degree in the secondary branches of a primary branch. Moreover, the same differences in rate of growth and reproduction as in *Pennaria* appear in different branches from apex to base of the colony or in the secondary branches of any branch, in other words the same relations of dominance and subordination exist in the two growth forms as wholes.

The chief difference between the two forms consists in the fact that in the monopodial *Pennaria* the terminal hydranth maintains its dominance over later buds and therefore remains terminal, while in the sympodial *Obelia* the latest bud becomes

dominant and displaces the former terminal hydranth. In this connection it is of interest to note that the *Obelia* bud grows for some time before it differentiates into a hydranth (Fig. 10). During this period of growth it is the seat of intense physiological activity and shows a high susceptibility, which decreases as the hydranth develops, and it is during this period that it displaces the former apical hydranth. In *Pennaria* the bud differentiates into a hydranth at once with but little growth in length (Fig. 7). That these differences in behavior are associated in some way with the differences in growth form cannot be doubted, and it seems probable that they may be the determining factor. Since dominance in axial relations has been shown to be fundamentally associated with differences is intensity of metabolic activity the intense growth activity of the bud of *Obelia* and similar forms probably enables it to dominate and displace the former apical bud which has completed its period of rapid growth and is undergoing differentiation into a hydranth. In *Pennaria*, on the other hand, where the bud differentiates with very little growth, the intensity of its activity decreases from an early stage and is apparently less at any stage than in the *Obelia* bud, consequently it is unable to alter existing axial relations and becomes a subordinate member of the complex. This interpretation is offered merely as a suggestion, based on the data at hand. Whether it is correct or not can probably be determined only by experimental work such as has been done with plants on control and modification of the relations of dominance and subordination by inhibition or removal of certain apical regions, but such work offers difficulties at present.

The necessity of using freshly collected material for the study of the regional differences in susceptibility, the colony gradients, as well as for other work on susceptibility may once more be emphasized. In colonies kept in the laboratory over night the regional differences have often disappeared or are reversed in the colony or branch.

That there is a real difference in physiological condition in different regions of the colony is indicated by the fact that the medusa buds in *Pennaria* and *Bougainvillea* and the gonozoöids in *Obelia* appear first in the most basal regions of the most basal

branches, *i.e.*, the regions where the susceptibility is on the average lower than anywhere else in the colony. From this region the localization of developing medusa buds and gonozoöids gradually progresses apically in the colony as a whole and in each branch. Moreover, at least many hydranths of *Pennaria*, after producing medusa buds for a time, degenerate and may be replaced by new ones, but whether such degeneration always follows medusa bud-formation, it is impossible to say. In *Obelia* the hydranths, which in this form have no direct part in the production of medusae, undergo disintegration or resorption, at least very frequently in regions of the colony basal to the level of gonozoöid formation. New hydranths may develop in place of the old, so that this loss of hydranths in relation to gonozoöid formation may readily escape notice, unless carefully looked for.

Evidently the physiological condition which determines the development of medusa buds and gonozoöids is attained in the basal regions of the colony earlier than elsewhere, and gradually progresses apically in the colony, followed by a zone in which at least many of the hydranths die or undergo resorption. This region where medusa buds or gonozoöids make their first appearance is the region where rate of growth and frequency of hydranth buds has been least and therefore physiological senescence has progressed most rapidly. In the sympodial colony of *Obelia* these regions are the oldest in time as well as physiologically, while in the monopodial *Pennaria* and *Bougainvillea* they are the oldest in time except the apical hydranths, but physiologically are the oldest of all, apparently because senescence has been retarded in the apical hydranths by the higher frequency of hydranth reproduction. There can be no doubt that the appearance of medusa buds or gonozoöids in a colony is associated with the attainment of a certain stage of progressive development and physiological senescence, and the facts presented above, with their interpretation, throw some light on the problem of this first appearance in basal regions and gradual progression apically in the colony.

In various algæ of similar growth form to the hydroids the specialized reproductive organs, *e.g.*, the cystocarps in numerous

species, the stichidia in *Dasya* and related forms, make their first appearance in the basal regions of the thallus and the zone of their development progresses apically, exactly as in the hydroids. Moreover, in the algæ as in the hydroids, the basal regions are the regions of lowest susceptibility and the oldest physiologically (Child, '16 *a*, *b*). That this similarity of localization of specialized reproductive parts, as well as the similarity of growth form in algæ and hydroids is associated with the gradations in physiological condition, of which the susceptibility relations are an indicator, cannot be doubted. And finally, it may be pointed out once more that this similarity in growth form, susceptibility relations and localization of specialized reproductive processes in algæ and many other plants and hydroids must depend upon some physiological factor which is independent of the specific differences in protoplasmic constitution of the different forms and which must therefore be quantitative in character. In short, all the facts force us to recognize the existence of a quantitative gradation in physiological condition, in rate of fundamental metabolism and the associated protoplasmic conditions, as the basis of the axial relations, of which growth form, susceptibility and localization of parts are various expressions.

Concerning the nature of the relation of dominance and subordination which has long been known to exist in the plants and is evidently present in the hydroids also, it need only be said here that the conception of dominance by means of inhibiting, formative or other specific substances accounts neither for the origin nor for the maintenance of these relations. In order that one region may produce substances that inhibit another region, the two regions must already be different, *i.e.*, in order that such chemical relations may exist along a physiological axis, the axis must already be established and certain differences be present. Moreover, if one apical region produces substances which inhibit other apical regions, the one region must be immune to the substances which it produces, while the other regions are not, that is to say differences must already exist between the different apical regions before one can inhibit another without itself being inhibited, and if differences already exist, the assump-

tion of the existence of inhibiting substances becomes wholly unnecessary. And finally, the definite and characteristic spatial relations between different axes in such a complex as the multiaxial plant and the hydroid cannot be accounted for in terms of the production and transportation of inhibiting or other substances. No sort of transportative relation can be conceived which affords an adequate interpretation of the facts. Only on the basis of transmission in protoplasm is it possible to account for both origin and maintenance of the relations which are demonstrated to be present in the organism. Of course after parts have become qualitatively different, more or less specific chemical relations undoubtedly exist between them, but these are results of physiological integration, not its determining conditions. Further discussion is postponed until additional evidence along other lines is presented.

Gradients in Staining with Vital Dyes.

When neutral red or methylene blue is used in determining differences in susceptibility, it is found that while these dyes enter all cells of the hydranth readily, differences in rate of staining appear in the earlier stages of the process. These differences show the same relations to the axes and to stage of development as the differences in susceptibility. The axial gradients in rate of staining indicate the existence either of differences in permeability to the dyes or of differences in the rate at which they are adsorbed or otherwise taken up by the cell constituents, or perhaps differences of both sorts. That the dyes are held in some way within the cells is evident from the fact that they accumulate there until their concentration is far higher than outside and do not pass out to an appreciable degree when the animals are returned to water. Similar differences in rate of staining with vital dyes, showing in general the same regional relations as the differences in susceptibility, have been noted in many other forms, both plant and animal, but it is evident that in the hydroids, as elsewhere, the differences in susceptibility to the dyes, as measured by time of death, cannot be due alone to the fact that the dyes enter certain cells more rapidly than others. Most of these differences in rate of staining, *e.g.*, those in dif-

ferent parts of the hydranth are slight or only temporarily visible in the earlier stages of staining, but the more extreme differences, such as those between hydranths and stems, remain visible for a longer time, in fact the hydranths may be killed by the stain before the stem is very deeply stained. In general, however, the differences which appear at first tend to become equalized as staining progresses. Apparently the rate of accumulation of the dye decreases most rapidly in those cells which at first stain most rapidly, so that the early differences in rate of staining are obliterated, at least to a large extent, by the action of the dye on the cells.

Nevertheless the differences in susceptibility, as indicated by survival time are very marked in these dyes, even in regions where the staining appears to be uniform in degree. The early differences in rate of staining are evidently not alone responsible for the differences in susceptibility. In fact it is much more probable, as has been suggested elsewhere (Child, '16*b*, Child and Hyman, '19), that the conditions indicated by the differences in staining rate, whether differences in permeability, adsorption or some other factor, are simply one aspect of the differences in fundamental physiological condition which determine differences in susceptibility. The facts of acclimation briefly referred to below, so far as they concern the vital dyes, show even more clearly that the differences in physiological effect are not due to the initial differences in rate of staining, for parts which stain at first more rapidly show a greater capacity for acclimation than less rapidly staining parts.

As death approaches in cells stained with neutral red, a distinct change in the color of the dye toward the acid side occurs, *i.e.*, the cell contents become distinctly acid shortly before disintegration. This change in color of neutral red occurs quite independently of the nature of the killing agent, which may, for example, be neutral red itself, an acid, KNC which is alkaline in solution, etc. Of course as soon as disintegration occurs the color of the neutral red is determined by the hydrogen ion concentration of the entering solution. The increase in acidity preceding death is sufficient to be clearly visible and in elongated axiate parts, *e.g.*, the tentacle or hydranth body, a gradient in

acidity preceeds the disintegration gradient. Similar increase in acidity preceding death has been observed in the cells of many algæ (Child, '16 *a*, *b*, '17). In many species of algæ this gradient in acidity is distinct within the length of a single elongated cell, even though the staining may have been quite uniform up to this stage. The axial relations of this increase in acidity are of course the same as those of the disintegrative changes and the color change of the dye may itself be used to a considerable extent as a criterion of susceptibility.

Acclimation.

Presentation of certain data which bear more or less directly upon the problem of acclimation, *e.g.*, the resorption and redevelopment of hydranths, the transformation of apical regions from hydranth production to stolon production and the reverse, must be deferred to another paper. For the present it may be noted that in low concentrations of various agents, KNC *m*/50,000–*m*/20,000, HCl *m*/2,000–*m*/1,500, LlCl *m*/50, ethyl urethane *m*/2,000–*m*/500, $MgSO_4$ *m*/500, neutral red and methylene blue, indications of differential acclimation appear. In the single hydranth under such conditions the body may die before the tentacles, but in most hydroids the hydranths undergo either disintegration or resorption so rapidly under slightly depressing conditions in the laboratory that the investigation of differential acclimation in the single hydranth is difficult.

Attention has been chiefly directed, however, to the regional differences in acclimation in the colony as a whole, which are very definite and characteristic. In general, the physiologically older hydranth shows less capacity than the younger hydranth or the bud for acclimation to slight inhibitions, consequently, in the course of a few days under such conditions, the older hydranths disintegrate or undergo resorption, while the younger persist or are affected to a less degree or less rapidly. Since the order of arrangement of hydranths of different physiological age along each axis and in the colony as a whole is definite and characteristic for the species (pp. 112–117), the regional differences in acclimation to low concentrations are in general the same as the differences in susceptibility to lethal concentrations, that is,

those zoöids which are most susceptible to the lethal concentrations, show the greatest capacity for acclimation to a certain range of low concentrations.

Conclusion and Summary.

The significant points in the experimental data are; first, that the susceptibility relations are definite, not merely for the single tentacle, hydranth and medusa bud, but for the single axis as a whole and for the axial complexes which result from branching and finally for the whole colony; second that they are similar to the relations observed in other organisms with similar growth forms, *e.g.*, the algæ. In general, the more apical regions of the physiologically younger axes of a complex, which are obviously the regions of most intense metabolism, are most susceptible to the higher concentrations or intensities and also most capable of becoming acclimated to slightly inhibiting conditions. These differences in susceptibility and capacity for acclimation are manifestly associated with quantitative differences in physiological condition, which are fundamental characteristics of physiological axes. The similarity of susceptibility relations in algæ and hydroids of similar growth form, indicates, if it does not demonstrate their independence of the specific differences in algal and hydroid protoplasms and therefore their essentially quantitative character. In spite of the differences in rate of staining with vital dyes, it is evident that differences in susceptibility cannot depend solely upon differences in permeability of limiting surfaces, though such differences in permeability are doubtless associated with the conditions on which susceptibility depends. Regional or axial differences in permeability are themselves dependent upon the fact that the membranes are living and therefore metabolically active, and upon the possibility of quantitative differences in this living condition. Some substances enter cells only as they produce irreversible changes in the living surface, while others enter all cells readily without appreciable injury to the surfaces, yet to both groups of substances the axial and regional differences in susceptibility are in general similar.

These data on susceptibility in the hydroids are in complete

agreement with other data on both animals and plants and constitute another bit of evidence in support of the conclusion that physiological axes are primarily gradients in rate of fundamental dynamic processes and in associated physical and structural conditions in living protoplasm.

The most important facts are summarized as follows:

1. In the hydranths of *Pennaria*, *Tubularia*, *Bougainvillea*, *Obelia*, *Gonothyria* and various other forms, the susceptibility to lethal concentrations of at least many external agents is greatest in the apical region of each tentacle and of the hydranth body and decreases basipetally.

2. In the elongated medusa bud of *Pennaria* the susceptibility decreases basipetally from the apical region.

3. Stems are in general much less susceptible than the more highly specialized structures, though the perisarc may act as a protective covering to some degree. At least in the more apical regions of axes the susceptibility of the stem also decreases basipetally, but in the more basal regions irregularities may appear.

4. Physiologically older zoöids or medusa buds are less susceptible than younger, except in certain cases where the development of motility increases susceptibility to such an extent as to overbalance the age differences.

5. Except in the earlier stages the medusa buds of *Pennaria* are less susceptible to lethal concentrations than the hydranth bodies from which they arise, and the gonophores and gonozoöids of other forms show the same relations.

6. In the colony as a whole and in each axial complex the average susceptibility of well developed hydranths decreases basipetally, but certain characteristic differences in axial relations exist in the monopodial (*Pennaria*) and the sympodial type (*Obelia*).

7. The medusa buds of *Pennaria* and *Bougainvillea* and the gonozoöids of *Obelia*, etc., appear first on those hydranths or in those regions of the colony where the susceptibility to lethal concentrations of agents is least, *i.e.*, in the basal regions of the more basal branches. From these regions the zone of development of these parts gradually progresses apically as the different levels successively attain the physiological condition which determines their development.

8. The very close relation between susceptibility and physiological condition is indicated by the fact that in various species the characteristic gradients and regional differences in susceptibility often disappear or are reversed after a day or two, or in some cases, after a few hours under laboratory conditions.

9. In general the regions which are more susceptible to lethal concentrations are more capable of acclimation to a certain range of lower concentrations.

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LOCOMOTION IN TWO SPECIES OF THE GASTROPOD GENUS ALECTRION WITH OBSERVATIONS ON THE BEHAVIOR OF PEDAL CILIA.

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INTRODUCTION.

In a paper on locomotion in gastropods Parker ('11) has pointed out that the mud snail, *Alectrion* (*Ilyanassa*) *obsoleta* Say, exhibits no rhythmic pedal movements such as are readily observed in most snails, and concludes that progression is accomplished by arhythmic muscular activity. He writes: "The movement of the foot of *Ilyanassa* has a most striking resemblance to that of the foot of a planarian in which cilia may be the chief motor organs, but on testing the foot of *Ilyanassa* with carmine suspended in sea water, not the least evidence of cilia could be discovered." In subsequent accounts of gastropod locomotion *Alectrion obsoleta* has been cited as representing the only definitely known case of arhythmic movement. Recently, however, Crozier ('19) has reported another example, that of *Conus agassizii* from Bermuda.

In the course of studies on the reactions of *Alectrion* I became interested in the locomotion of the animal and tested the foot for cilia by dropping carmine grains on its ventral surface. Much to my surprise the carmine was carried down the foot, and when the latter was examined under the microscope the under surface was found to be covered with cilia. Another related species, *Alectrion* (*Tritia*) *trivittata* Say, which showed no evidence of pedal waves likewise proved to have a ciliated foot.

That cilia occur on the feet of certain species of gastropods has been known for some time, but that they function as locomotor organs, however, has been questioned, and I believe that no detailed study of their behavior has been made. Olmsted ('17) has recently reported finding cilia on the feet of three species of marine snails from Bermuda, which showed no evidence of pedal

waves, and attributes their locomotion to ciliary action. Since, however, it is held that progression may be brought about by muscular activity without the appearance of pedal waves (arhythmic locomotion), the presence of cilia and the absence of waves does not signify necessarily that locomotion is accomplished by cilia. It is conceivable that pedal cilia have some other function, such as freeing the foot from foreign material or distributing mucus. If some such conception of ciliary function had not been held by students of gastropods, movement by ciliary action should have been recognized long ago as unquestionably constituting one type of locomotion in these animals. Walter in 1906 described the mechanism of locomotion in the pond snail, *Lymnæus elodes* Say, as consisting of "a complex muscular foot clothed on the ventral surface with cilia which act on a mucus track," and did not record finding any pedal waves, yet this case, as far as I know, has never been cited as substantiating the theory that gastropod locomotion may sometimes be effected by cilia.

In view of these facts it seemed desirable to investigate in some detail the behavior of the cilia on the feet of *Alectrion* in the hope of throwing light upon the possible relation between ciliary action and locomotion. The work was carried on at the Marine Biological Laboratory at Woods Hole, and I wish to express my gratitude to the director, Dr. Frank R. Lillie, for his kindness in granting me the facilities of the laboratory.

Ciliary Behavior.

Alectrion trivittata.—The foot of *Alectrion trivittata* in an individual of medium size measures 10 or 12 mm. in length by 4 or 5 mm. in width. The anterior end is truncated and auriculate. Back of the lateral, pointed processes the foot is narrowed somewhat before it expands and tapers toward the posterior end, which is bifurcated and bears two small tentacles. It is more slender than the foot of *Alectrion obsoleta* and considerably thinner, a feature which makes the species a favorable one for the study of ciliary activity under the microscope.

As soon as it was recognized that the under surface of the foot was ciliated the question arose: Were the cilia active during

locomotion, and if so, when the animal came to rest, did ciliary movement stop, or did it continue without effecting forward movement? That the cilia did not beat continuously was soon demonstrated. A support for the snail was made by fastening with "sticky-wax" a short glass tube to the bottom of a glass dish containing sea water. The snail's shell was then stuck upside down to the end of the pedestal so that the ventral surface of the foot when fully expanded was at the level of the water. In this position the animal usually exhibited righting movements for a time, but soon spread out its foot on the surface film of the water. Often the flicker of the cilia could be seen with the naked eye and readily observed under a hand lens. Grains of carmine were swept down the foot by ciliary activity. At other times, however, the cilia were quiescent and carmine grains remained on the foot where they were dropped. When a snail resting in this position was stimulated by touching one of its tentacles with a piece of fish meat, the proboscis was protruded and the cilia began beating. The proboscis was often worked over the surface of the foot and at that time the ciliary movement was greatly reduced or stopped. Usually after the proboscis was withdrawn the cilia showed vigorous activity, the foot was stretched out its full length, and the animal appeared as it did when moving upside down on the surface film of the water, a form of progression often exhibited when free in the aquarium. In the later situation its behavior was essentially like that when fixed in the position just described. As it moved along the surface of the water the cilia could be seen in motion and carmine grains were swept with the mucus down the foot. Frequently the proboscis was extended and moved about in various directions, and at this time ciliary activity and locomotion ceased.

By placing the dish, in which an inverted snail was fastened, on the stage of a microscope, and by using a low power objective the fringe of long cilia projecting from the anterior border of the foot could be clearly observed. In one instance when the animal was becoming quiet after feeding, the cilia could be seen beating slowly near the tip of one of the anterior lateral foot processes, whereas other cilia nearer the median position were

motionless. Moving cilia were observed slowing down and coming to rest, and passive ones becoming slightly active. Suddenly all the cilia under observation began to beat at high speed, when compared with the slower movement first recorded, and the foot process moved out of the field of vision. The snail was now showing its normal surface "swimming" activity.

The relation of ciliary movement to locomotion was finally studied by observing under the microscope snails which were moving on the under side of a glass slide placed across a dish full of sea water. A resting animal showed the cilia on the anterior border and ventral surface of the foot passive. Sometimes, when the snail was feeding, one of the lateral foot processes was moved or contracted slightly and some local beating of the cilia of short duration occurred. When the animal was moving, all the cilia which could be seen were active, and moreover, the rate at which they beat was distinctly correlated with the speed of locomotion. Rapidly beating cilia decreased their rate of movement and came to rest when the animal did, and became active again when locomotion began. Very satisfactory observations were made by placing the snails on the under surface of a slide, which had been smeared with fish meat, and allowing them to feed in this inverted position out of the water. Under these conditions they frequently progressed short distances and stopped, and the cilia in turn were seen to start beating, to continue slowly while the animal moved, and then to come to rest. When the rate of locomotion was increased, the cilia beat so rapidly that they could only with difficulty be seen at all.

A microscopic examination of the cilia of snails moving and floating at the surface of the water confirmed the observations already reported.

Alectrion obsoleta.—The foot of *Alectrion obsoleta* resembles that of *Alectrion trivittata* but is thicker, not so long in proportion to its width, rounded at the posterior end and lacks posterior tentacles. The behavior of the cilia were studied by some of the methods described in connection with the work on *Alectrion trivittata*, and the results obtained were so closely similar it is unnecessary to report them in detail. The thicker foot of the

mud snail made microscopic observation of the cilia on the ventral surface more difficult, but they could be seen when beating, and those along the anterior border were clearly visible at all times.

A comparison of the habits of the two species brought out one striking difference. Whereas *Alectrion trivittata* often floats or moves upside down at the surface of the water after the manner of certain fresh-water gastropods, *Alectrion obsoleta* was never observed to do so. Dimon ('05) in her monograph on the latter species states that young individuals may float on the surface but that the older ones very rarely do, and then only under unusual circumstances, and concludes that the shell of the adult is too heavy to permit it. It was impossible therefore to observe the behavior of the cilia on the foot of a freely floating mud snail.

The study of the two species of *Alectrion* shows unquestionably that beating cilia are characteristic of a moving snail, and that when the animal is at rest the cilia are motionless or exhibit only local activity. It is equally clear that the rate of ciliary movement is correlated with that of locomotion.

The reason why Parker, in the investigation referred to, failed to find cilia on the foot of *Alectrion obsoleta* was undoubtably because when he placed carmine on them they happened to be quiescent. Only the unusual behavior of the cilia prevented their immediate discovery by the test he applied.

Comparison of Locomotor with Ciliary Rates.

The foregoing observations are strongly in favor of the view that progression in *Alectrion* is accomplished by ciliary action. In order to test these conclusions further, the rate of locomotion was compared with the rate at which grains of carmine were driven over the surface of the foot by the beating cilia. The latter may be termed the ciliary rate. By the use of a stop-watch it was an easy matter to secure a series of records indicating the speed at which the snails traveled a distance of 5 mm. over the bottom of a glass dish containing sea water. In order to obtain a ciliary rate, the snail was fixed upside down on a support so that its foot when expanded was at the surface of the water.

A millimeter rule was fastened above the foot. Particles of dry carmine were then dropped on the water and as they were driven with the mucus down the foot by the active cilia, it was possible to record their speed over a distance of 5 mm. with considerable accuracy.

For some time all attempts at securing the ciliary rate for *Alectrion obsoleta* were unsuccessful. The snails either continued in their efforts to right themselves or else failed to expand their feet at the surface of the water, as *Alectrion trivittata* did with little hesitancy. When about to abandon the experiment some individuals not fully grown were tried, and their behavior was so satisfactory the desired records were readily obtained. In one instance continuous ciliary movement was watched for about three quarters of an hour. It is quite possible that the difficulty encountered with the older individuals was due to the fact that in the adult state the species does not have the habit of surface floating, whereas the younger animals adapted themselves to the inverted position more readily because they had more recently abandoned it under natural conditions.

The results of the tests made upon *Alectrion obsoleta* are shown in Table I. They indicate that the average locomotor rate is practically identical with the rate at which carmine grains are driven along the foot by the beating cilia.

TABLE I.

Alectrion obsoleta.

	Locomotor Rate. (Rate at which Snails Moved a Distance of 5 Mm.).			*Ciliary Rate.* (Rate at which Carmine Grains were Carried a Distance of 5 Mm. Over Ciliated Foot.)	
Animal numbe	1	2	6 individuals	1	2
Number of trials	25	25	10 each	25	25
Average time in seconds	2.4	2.9	3.	2.5	3.

Animal number one proved to be a faster moving individual than any of the others timed, and it will be noted that the average ciliary rate is also fast. Considering the fact that the cilia are subject to great variation in rapidity of movement, the correspondence in rates is closer than might be expected, and to my mind affords conclusive evidence that locomotion is entirely dependent on the action of cilia.

Turning now to Table II it will be seen that the average rate of locomotion of *Alectrion trivittata* is twice as fast as that of the mud snail. If one watches for a moment the two species moving about in an aquarium, he does not require the use of a stopwatch to be convinced of this difference. The average ciliary rate of

TABLE II.

Alectrion trivittata.

	Locomotor Rate. (Rate at which Snails Moved a Distance of 5 Mm.).			*Ciliary Rate.* (Rate at which Carmine Grains were Carried a Distance of 5 Mm. Over Ciliated Foot.)	
Animal number	1	2	6 individuals	1	2
Number of trials	10	10	10 each	10	10
Average time in seconds	1.8	1.3	1.5	2.7	2.1

Alectrion trivittata (2.3 seconds in eighty trials) is also faster than that of the mud snail, but slower than its own locomotor rate. The latter conclusion is based on many more trials than are recorded in the table. Does this mean that some arhythmic muscular operation is associated with ciliary movement and is responsible for the difference between the two rates, or do the cilia tend to beat faster when the snail is moving on its foot than they do when it is fastened on its back? An investigation of the locomotion of small pieces of the foot led me to believe that the latter explanation is the true one.

The behavior of the cilia on an excised foot will be described later in detail, and it is only necessary to record here that fragments of the foot a few millimeters long move at times rapidly over the substrate by ciliary action. The direction of their movements, however, was so uncertain it was necessary to time their speed over a distance of 2.5 mm. Some of these pedal fragments frequently moved at the rate of 5 mm. in 1.6 seconds and occasionally in 1.2 seconds, or faster than the average rate of locomotion recorded for six snails in Table II. In the case of these small pieces of the snail's foot there was no possibility that locomotion was in any direct way brought about by muscular movements. It was entirely the result of ciliary action, and the resultant speed was closely similar to the usual locomotor rate of the species. Under the microscope the cilia on the frag-

ments were sometimes seen at rest, again beating slowly and frequently at a high rate of speed, all accounting perfectly for the erratic movements of the fragments. There is no evidence that the locomotor mechanism of *Alectrion trivittata* contains any important factor not represented in that of *Alectrion obsoleta*, and I believe that progression in both species is accomplished by cilia.

If, as it appears, the rapidity of ciliary movement in *Alectrion trivittata* tends to be reduced when the animal is held with the ventral side of its foot at the surface of the water, some explanation of this peculiarity should be sought. I believe it is to be found in its habit of surface floating and moving, one not shared by adult mud snails. Observation of the snails' movements at the surface of the water gives one the immediate impression that this form of activity is a decidedly leisurely one associated with feeding. A snail may remain floating with extended proboscis for a long time, and when it moves, it is often for short distances in an apparently aimless way. At such times the cilia probably beat more slowly than they do when the animal is moving in a definite course over a substrate, as it was when the locomotor rates were taken.[1] When the snail is fixed to a support with its expanded foot at the surface of the water, its normal floating position is closely simulated. It accepts the inverted position often without showing any extended disturbance, and always exhibits the feeding reaction when stimulated with fish meat. It would not be surprising, therefore, if the behavior of the cilia were similar under these two conditions, and I believe that a somewhat reduced ciliary activity is characteristic of both.

Ciliary Control.

In connection with the investigations described in the preceding pages there developed the exceedingly interesting problem of ciliary control. Doubtless it has occurred to the reader that the

[1] That snails move more slowly at the surface of the water than they do over a glass surface is perfectly clear, the average speed of locomotion in the former situation, based on thirty-four trials, being at the rate of 5 mm. in 2.7 seconds. Drawing conclusions, however, on the rapidity of ciliary movement by direct comparison of these two locomotor rates may not be justified, for a substrate is not present in both cases.

behavior of the pedal cilia of *Alectrion* suggests that they have in some way been appropriated by the nervous system and are controlled directly or indirectly by nervous impulses. Although little more than a beginning has been made in the study of this phase of the problem, the results of certain experiments are sufficiently important to warrant a preliminary report at this time. They have been obtained for the most part from a study of ciliary behavior after the foot has been removed from the rest of the snail's body.

When the somewhat contracted, excised foot of *Alectrion trivittata* is placed in sea water and viewed under a microscope the greater number of cilia are seen to be motionless. If now the anterior margin of the foot is watched, it will be observed that at intervals slight contractions of the border occur accompanied by the beating of the anterior fringe of cilia over the area moved. This muscular movement is not extensive, but consists of a sudden withdrawal of a short segment of the pedal border so that for a moment it appears slightly indented, the contraction being immediately followed by an expansion which restores the normal contour of the foot. The ciliary activity is also but a momentary affair and is restricted to the contracted region. The cilia usually appear to beat either synchronously with the contraction or directly after it, but in certain instances ciliary movement seems to precede slightly the muscular movement. These contractions accompanied by local ciliary action sometimes continue for hours. Sooner or later, however, the twitching of the muscles becomes more vigorous and frequent, and the cilia fail to come to rest between contractions. Some of them may be beating continuously at the same time that others are still showing intermittent activity associated with muscle movements. Finally the cilia are in motion, not only along the entire anterior border and lateral processes of the foot, but also over the ventral surface, and in the latter position contractions are also going on. At this time the anterior cilia exhibit great variation in rapidity of movement. Occasionally they slow down nearly coming to rest in the intervals between contractions, and at other times move so fast that not the slightest flicker can be seen, the ciliary fringe appearing like a broad

hyaline membrane. This high rate of movement is always associated with muscular contractions. Why it was that *Alectrion trivittata* sometimes moved so swiftly was never fully understood until I had witnessed in this way the possibilities of ciliary motion.

The third stage of ciliary activity is seen as a rule a number of hours later, often about twenty-four hours after the foot is severed from the body. At this time all muscular movement has ceased and the whole foot has become expanded. The cilia are beating everywhere at moderate speed, and if a given area is watched, no variation in the rapidity of movement is observed. The contrast between this uniform rate of ciliary movement and the preceding highly variable one is very striking. The cilia continue beating until maceration sets in and the epithelium begins to break down.

Having determined from a series of tests that the ciliary activities described above occurred with remarkable constancy, a means was sought of controlling muscular contractions without directly affecting the cilia. Previous experience had shown that magnesium sulphate is an excellent anæsthetic for marine gastropods and one that appears to leave no undesirable effects. It proved to be equally valuable in the study of ciliary behavior. When a foot of *Alectrion trivittata* in which muscular movements are going on is placed in a ten per cent. solution of magnesium sulphate made up in sea water, all contractions cease in a few minutes and the foot remains passive. The cilia, however, which are vigorously beating when muscular activity subsides, continue to do so for many hours with only gradually decreasing speed, whereas those which are quiescent remain so. Moreover, if there is initial variation in the rate of ciliary movement in different parts of the anterior border of the foot, it is maintained, and the cilia beating the fastest remain active the longest time; but changes in the speed of ciliary movement over a given area, which are so striking when the foot is in sea water and muscular contractions are in progress, never occur after contractility is inhibited by magnesium sulphate. It is also clear that those cilia which are moving most rapidly, or with the least interruption, when the foot is in sea water tend to beat the fastest when

the foot is removed to a solution of magnesium sulphate. If now the foot is returned to sea water, muscular contractions reappear in a few minutes and ciliary behavior is just the same as it was before the transfer.

These experiments indicate that the pedal cilia are normally set into activity and their rate of beating is modified either by the contraction of underlying muscle fibers or by direct nervous impulses. The fact that cilia continue beating in a magnesium sulphate solution after muscle activity has disappeared shows too that they have the power like ordinary cilia of maintaining movement in the absence of extra-epithelial stimulation, and finally, since their movement persists until they become exhausted or the epithelium breaks down, it is evident that they depend on some extrinsic factor to bring them to rest. In this connection it should be recalled that a foot which has been in sea water for a long time, perhaps for twenty-four hours, shows the cilia everywhere moving at uniform speed over a given area in the complete absence of muscular movement. In the latter case all the cilia were stimulated to movement before nervous impulses and muscular contractility gradually disappeared, and their behavior is the same as it is after these activities are suddenly inhibited by magnesium sulphate. When a foot which exhibits no muscular contractions is placed in a ten per cent. solution of magnesium sulphate the most marked change noted is but a slight retardation in the rate of ciliary movement. A more concentrated solution may bring about a decided slowing down or even a cessation of ciliary action. This appears to be a direct effect upon the ciliated cells, which show normal activity again after the foot has been transferred to sea water.

During the course of one experiment I was surprised to note certain cilia beating in the magnesium sulphate solution which were quiescent after the foot was placed in the solution twenty-four hours previously. An examination of the foot showed that it had revived during its long immersion in the fluid, and that contractions, such as ordinarily accompany the movement of the cilia, were in progress. Another instance of the reoccurrence of muscular and ciliary activity was recorded in the case of a foot which had remained in a magnesium sulphate solution over twenty hours.

A few experiments were carried on in which the snail itself was placed in a solution of magnesium sulphate. The behavior of the cilia was essentially the same as it was when the foot was severed from the more dorsal parts of the body.

Although the ciliary activities on the excised foot of the mud snail were not studied so thoroughly as in the case of *Alectrion trivittata*, enough work was done to convince me that the ciliary mechanism of both species operates in the same way.

Finally a careful investigation was made of the relation between muscular and ciliary movements when the snail was fastened to a support with its foot expanded at the surface of the water. Here also the cilia along the anterior border were seen to start beating as muscle contractions occurred, and to decrease their rate of movement as the contractions subsided and stopped. The lateral foot processes were sometimes swung about and this movement was also accompanied by local ciliary activity. When the cilia were beating actively and continuously the anterior border of the foot showed a vibratory motion caused by a series of rapid contractions and expansions. In addition to the more local muscular contractions correlated with ciliary action, there sometimes occurred irregular foot movements of greater distribution which were unassociated with beating cilia. Movements of the latter type were also observed occasionally when the foot was removed from the body.

Conclusions.

The observations and experiments briefly recorded above lead me to believe that locomotion in *Alectrion* is the direct result of ciliary action and not arhythmic muscular movements, and the unusual behavior of the cilia points to the conclusion that they have been brought in one way or another under the control of the nervous system. The fact that a resting snail can be made to move its pedal cilia by stimulating the receptors on one of its tentacles with fish meat is in itself sufficient evidence of the controlling action of the nervous system. The muscular movements, which occur when cilia begin beating and which accompany vigorous ciliary activity, may be explained by assuming that efferent impulses arrive at the ciliated cells and muscles at

approximately the same time. It is possible, however, that nervous impulses travel only to the muscles, and that the latter in some way exert a stimulating influence on the overlying ciliated epithelium. Further investigation, both histological and physiological, is planned which, it is hoped, will throw more light on the problem.

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